US ARMY INSTITUTE OF SURGICAL RESEARCH

ANNUAL RESEARCH PROGRESS REPORT

FOR FISCAL YEAR 1986

1 OCTOBER 1985 - 30 SEPTEMBER 1986

PREPARED BY: US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

PREPARED FOR: US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORT DETRICK
FREDERICK, MARYLAND 21701-5012

1 October 1987

APPROVED FOR PUBLIC RELEASE. DISTRIBUTION UNLIMITED.

DESTROY THIS REPORT WHEN IT IS NO LONGER NEEDED. DO NOT RETURN IT TO THE ORIGINATOR.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to Army Regulation 70-25 and US Army Medical Research and Development Command Regulation 70-25 on the use of volunteers in research.

In conducting the research described in this report, the investigators adhered to the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals and with the Guide for the Care and Use of Laboratory Animals, National Institutes of Health Publication 86-23.

Citations of commerical organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

This publication was compiled and edited by Christine C. Davis, Research Protocol Coordinator, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200.

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp. Date Jun 30, 1986

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT APPROVED FOR PUBLIC RELEASE. DISTRIBUTION UNLIMITED.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION US Army Institute of Surgical Research 6b. OFFICE SYMBOL (If applicable) SGRD-USM	7a. NAME OF MONITORING ORGANIZATION US Army Medical Research and Development Command
6c. ADDRESS (City, State, and ZIP Code) Fort Sam Houston San Antonio, Texas 78234-6200	7b. ADDRESS (City, State, and ZIP Code) Fort Detrick Frederick, Maryland 21701-5012
8a. NAME OF FUNDING/SPONSORING ORGANIZATION 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO SEE PAGES IX AND X OF THIS REPORT FOR COMPLETE LISTING
11. TITLE (Include Security Classification) US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1986 (U)	
12. PERSONAL AUTHOR(S) PRUITT, Basil A. Jr.	
13a. TYPE OF REPORT Annual 13b TIME COVERED FROM 85/10/01 TO 86/09/30	14. DATE OF REPORT (Year, Month, Day) 1 October 1986 15. PAGE COUNT 474
16. SUPPLEMENTARY NOTATION	
17. COSATI CODES FIELD / GROUP / SUB-GROUP	18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
19. ABSTRACT (Continue on reverse if necessary and identify by block number)	
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	21 ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL COL Basil A. Pruitt, Jr., MD, FACS	22b TELEPHONE (Include Area Code) 512-221-2720 22c OFFICE SYMBOL SGRD-USZ

DD FORM 1473, 84 MAR 83 APR edition may be used until exhausted
All other editions are obsolete

SECURITY CLASSIFICATION OF THIS PAGE

DEPARTMENT OF THE ARMY
US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON, TEXAS 78234-6200

SGRD-USZ

1 October 1986

MEMORANDUM: SEE DISTRIBUTION

SUBJECT: US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1986

The US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1986 is forwarded under the provisions of OTSG Regulation 70-31 dated 2 April 1969.

Basil A. Pruitt, Jr.

Enclosure
as

BASIL A. PRUITT, JR., MD, FACS
Colonel, MC
Commander and Director

FOREWORD

The annual cost of health care and biomedical research in the United States has exceeded 10 percent of the gross national product since 1981 and has risen by more than 10-percent in each of the past five years. These increases in the cost of health care are attributable to increased utilization of such care in general, more frequent application of expensive technology, and increased employment of prolonged personnel-intensive treatment of critically ill and severely injured patients. The increases in the cost of biomedical research are attributable to the necessary involvement of multidisciplinary investigators and to increased equipment acquisition and replacement costs necessitated by rapid technological developments.

This Institute, in which both patient care and research activities are focused on the severely injured soldier, is particularly susceptible to such increases in costs and personnel requirements. The nadir of annual admissions occasioned by the modernization of the Institute's clinical facilities has been superseded by an increase in admissions during the current fiscal year, among which both the number and duration of care of critically ill patients has increased. Similarly, the hemodynamic, pulmonary, and immunologic studies reported in this volume have involved multidisciplinary research teams, the use of newly developed equipment, and extensive computer support. These increased professional requirements have produced discordances between supply and demand since the Institute's most recent manpower survey and transfers of positions to administrative units have resulted in a decrease in authorized positions, and the Institute's budget increases of less than nine percent in fiscal year 1985 and less than four percent in the current fiscal year have failed to maintain constant dollar support.

The effects of this erosion of available resources have been exaggerated by an increased number of aeromedical transfers of seriously burned patients that have consumed in excess of 5,000 manhours during the past two years and increased readiness-related training activities, i.e., in-house training of interns, residents, and reserve physicians, organization and presentation of Army short courses, participation in the C4 and C4-A Courses, presentation of symposia to reserve organizations, and participation in NATO workshops and Air Force Red Flag exercises. Clinical and research productivity, efficiency, and flexibility have been further compromised by critical space limitations and deterioration of laboratory facilities of World War II vintage.

The clinical and research accomplishments reported in this volume attest to the industry and professionalism by which the Institute staff have overcome the workload demand-resource supply discrepancy to advance our understanding of the pathophysiologic effects of injury in general and to improve the management and increase the survival of burn patients in particular. The professional output of this Institute directly benefits the injured soldier, provides discrete support for redress of the imbalance betwen the military relevance of combat casualty care and resource allocation, and fully justifies the planned construction of new clinical and laboratory facilities.

Basil A. Pruitt, Jr.

BASIL A. PRUITT, JR., MD, FACS
Colonel, MC
Commander and Director

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army of the Department of Defense.

Council of Teaching Hospitals Report, Volume 16, Number 6, August 1982, p 5.

Culliton BJ: Congress passes general NIH budget. Science 222:483-484, 1983.

TABLE OF CONTENTS

Page Number

PROJECT NUMBER 3S162772A874-00, APPLIED RESEARCH

CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS..... 1

COL William F. McManus, MD; COL Anton J. Jirka, MD; LTC Kevin J. Farrell, MD; LTC Khan Z. Shirani, MD; LTC George M. Vaughan, MD; LTC Donna W. Kyzar; MAJ Robert C. Allen, MD; MAJ Lawrence W. Lehrner, MD; MAJ James C. McKay, MD; MAJ Anne E. Missavage, MD; MAJ Steven I. Schmidt, MD; MAJ Anthony A. Smith, MD; MAJ Patricia S. Latona, RN; CPT David W. Mozingo, MD; CPT L. Willis Roberts, MD; CPT Gary R. Culbertson, MD; CPT Ralph D. Cook, MD; CPT Rosendo T. Gutierrez; CPT Stephen H. Luster; CPT Kathleen F. McCoy; CPT Alvis T. Perry, MD; CPT Kathryn E. Robertson, RN; CPT Richard G. Simmons, Jr.; CPT Nancy K. McLaurin; CPT Susan W. Wilson; and COL Basil A. Pruitt, Jr., MD

An Analysis of the Adaptation of Renal Function Following Burn Injury..... 32

MAJ James C. McKay, MD; Calvin R. Kennedy; Arthur D. Mason, Jr., MD; LTC George M. Vaughan, MD; and COL Basil A. Pruitt, Jr., MD

Anesthesiology..... 40

MAJ Steven I. Schmidt, MD; CPT Charles P. Kingsley, MD; COL William F. McManus, MD; and COL Basil A. Pruitt, Jr., MD

Antithrombin III Deficiency in Thermally Injured Patients..... 50

Steven M. Pratt, MD; MAJ Robert C. Allen, MD; MAJ Anthony A. Smith, MD; CPT Michael Berkland, MD; COL William F. McManus, MD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

Page Number

PROJECT NUMBER 3S162772A874-00, APPLIED RESEARCH

Chemical Burns..... 58

CPT David W. Mozingo, MD; MAJ Anthony A. Smith, MD; COL William F. McManus, MD; COL Basil A. Pruitt, Jr., MD; and Arthur D. Mason, Jr., MD

The Influence of Inhalation Injury and Pneumonia on Burn Mortality..... 75

LTC Khan Z. Shirani, MD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY..... 95

E-Z Derm™ and Biobrane - A Comparative Study... 96

Stephen M. Pratt, MD; COL William F. McManus, MD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

The Influence Upon Electrolyte and Water Balance of Tap Water Showers in Burned Patients..... 105

Marisa R. Herson, MD; CPT Bryan S. Jordan, RN; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

Reduction of Blood Loss During Tangential Excision of Burn Wounds..... 110

Stephen M. Pratt, MD; COL William F. McManus, MD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

A Study to Evaluate the Effectiveness and Safety of Artificial Skin in the Treatment of Third Degree Flame or Scald Injuries..... 115

Stephen M. Pratt, MD; CPT Bryan S. Jordan, RN; COL William F. McManus, MD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

Page Number

PROJECT NUMBER 3S162772A874-00, APPLIED RESEARCH

5% Aqueous Sulfamylon Soaks Used in Topical Treatment of Burned Soldiers..... 135

COL William F. McManus, MD, and COL Basil A. Pruitt, Jr., MD

STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN BURN INJURY..... 138

Nyctohemeral Rhythm in Melatonin Response to Isoproterenol in Syrian hamsters..... 140

LTC George M. Vaughan, MD; COL Basil A. Pruitt, Jr., MD; and Arthur D. Mason, Jr., MD

Page Number

PROJECT NUMBER 3M161102BS10-00, BASIC RESEARCH

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY..... 154

Albert T. McManus, PhD; Jack R. Henderson, PhD; SSG Timothy E. Lawson; SGT Charles E. Guymon; SSG Aldo H. Reyes, SSG Kenneth Hawthorne; SGT Anne Lasko; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

Effectiveness of Mafenide Acetate and Silver Sulfadiazine in the Scalded Mouse Model..... 199

CPT George B. Stover, MS; Albert T. McManus, PhD; LTC Gene B. Hubbard, DVM, MS; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

A Multicenter Open Study of the Efficacy, Safety, and Tolerance of PRIMAXIN® (Previously Referred to as MK0787/MK0791 and Thienamycin Formamidine/Potentiator) in the Parenteral Therapy of Infection Caused by Pathogenic Bacteria in Hospitalized Patients..... 212

CPT Gary R. Culbertson, MD; Albert T. McManus, PhD; CPT Patrick A. Conarro, MD; COL William F. McManus, MD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

Pharmokinetic Study of Amikacin in Burned Patients..... 220

MAJ James C. McKay, MD; Albert T. McManus, PhD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

Therapeutic and Prophylactic Effects of Silver-Nylon Dressings with Weak Direct Current on Experimental *Proteus mirabilis* Burn Wound Sepsis..... 224

Chi-Sing Chu, MD; Albert T. McManus, PhD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS..... 234

Page Number

PROJECT NUMBER 3M161102BS10-00, BASIC RESEARCH

ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS.. 235

Characterization of Biochemical Indicators of Infection in the Thermally Injured..... 244

LTC David G. Burleson, PhD; Kuang-Tzu D. Lin, MD; Avery A. Johnson, BS; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

Preliminary Studies of Lymphoid Subpopulations in a Burned Animal Model..... 257

LTC David G. Burleson, PhD; Albert T. McManus, PhD; Ysidro Villareal; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

Therapy with IgG and T_4 in Burn Patients..... 266

MAJ Anne E. Missavage, MD; LTC Khan Z. Shirani, MD; LTC George M. Vaughan, MD; Albert T. McManus, PhD; LTC David G. Burleson, PhD; COL William F. McManus, MD; COL Basil A. Pruitt, Jr., MD; and Arthur D. Mason, Jr., MD

ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY.. 278

Thyroid Hormone Economy After Burns - Two Patterns of Thyrotropin Alteration and Lack of Change in Thyronine Binding Globulin..... 280

LTC George M. Vaughan, MD; Richard A. Becker, MD; LTC Khan Z. Shirani, MD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

INEQUALITY OF VA/Q RATIOS FOLLOWING SMOKE INHALATION INJURY AND THE EFFECT OF ANGIOTENSIN ANALOGUES..... 299

PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL..... 300

Page Number

PROJECT NUMBER 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Cardiovascular and Endocrine Sequelae of Burn Resuscitation..... 302

Hemodynamic Effects of Controlled Pure Plasma Blood Loss in Sheep..... 304

Marisa R. Herson, MD, and Arthur D. Mason, Jr., MD

Cellular Host Defense Function After Thermal Injury: Assessment by Flow Cytometry of Peripheral Blood Cells..... 336

LTC David G. Burleson, PhD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

A Study of Biochemical Changes in the Cellular Environment of Tissue of the *in vivo* Partial-Thickness Rat Burn Wound..... 355

Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model..... 356

CPT Ronald L. Shippee, PhD; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

Derivative Spectroscopy Chemiluminigenic Probing of Granulocyte Redox Function in Healthy Controls and Burn Patients..... 383

Robert C. Allen, MD, PhD; David F. Jadwin, DO, Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

Cultured Keratinocytes as Epithelial Grafts for Burned Soldiers..... 402

Page Number

PROJECT NUMBER 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

Inequality of VA/Q Ratios Following Smoke Inhalation Injury and the Effect of Angiotensin Analogues..... 403

Effects of (1-Sarcosin, 8-Isoleucine) Angiotensin II on Smoke Inhalation Injury..... 404

Takeshi Shimazu, MD; LTC George M. Vaughan, MD; Tetsuo Yukioka, MD; Tsuyoshi Sugimoto, MD; LTC Gene B. Hubbard, DVM, MS; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

Platelet-Activating Factor (PAF) in a Sheep Model of Smoke Inhalation Injury - A Preliminary Report..... 422

Takeshi Shimazu, MD; Hisashi Ikeuchi, MD; Raj Kumar, PhD; Donald J. Hanahan, PhD; LTC Gene B. Hubbard, DVM, MS; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

Time Course of VA/Q Alterations Following Smoke Inhalation Injury in a Sheep Model..... 435

Takeshi Shimazu, MD; Avery A. Johnson, Jr., BS; LTC Gene B. Hubbard, DVM, MS; Arthur D. Mason, Jr., MD; and COL Basil A. Pruitt, Jr., MD

	Page Number
PRESENTATIONS.....	446
PUBLICATIONS.....	454
MOTION PICTURES.....	456
DISTRIBUTION LIST.....	457

FISCAL YEAR 1986		FISCAL YEAR 1987			
Project Number	Work Unit Number	Project Number	Work Unit Number		PAGE NUMBER
3S162772A874				APPLIED RESEARCH	
3S162772A874	164	3S162772A874	164	Clinical Operation, Center for Treatment of Burned Soldiers	1
3S162772A874	162	3S162772A874	162	Evaluation of Burn Wound Care in Troops with Burn Injury	95
3S162772A874	163	3S162772A874	163	Studies of the Neuroendocrine Abnormalities of Burned Soldiers	138

	FISCAL YEAR 1986		FISCAL YEAR 1987			
	Project Number	Work Unit Number	Project Number	Work Unit Number		PAGE NUMBER
	3M161102BS10				BASIC RESEARCH	
	3M161102BS10	301	3M161102BS10	301	Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injury	154
	3M161102BS10	302			The Study of Metabolism and Nutritional Effects of Burn Injury in Soldiers	234
	3M161102BS10	303	3M161102BS10	303	Alteration of Host Resistance in Burned Soldiers	235
	3M161102BS10	304	3M161102BS10	304	Role of Thyroid Hormones in Burn Pathophysiology	278
×	3A161101A91C	089	3M161102BS10	305	Inequality of VA/Q Ratios Following Smoke Inhalation Injury and the Effect of Angiotensin Analogues	299
	3A161101A91C	078	3M161102BS10	306	Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model	300

FISCAL YEAR 1986		FISCAL YEAR 1987			
Project Number	Work Unit Number	Project Number	Work Unit Number		PAGE NUMBER
3A161101A91C				IN-HOUSE LABORATORY INDEPENDENT RESEARCH	
3A161101A91C	075	3A161101A91C	075	Cardiovascular and Endocrine Sequelae of Burn Resuscitation	302
		3A161101A91C	076	Cellular Host Defense Function After Thermal Injury: Assessment by Flow Cytometry of Peripheral Blood Cells	336
		3A161101A91C	077	A Study of Biochemical Changes in the Cellular Environment of Tissue of the in vivo Partial-Thickness Rat Burn Wound	355
3A161101A91C	078	3M161102BS10	306	Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model	356
3A161101A91C	079	3A161101A91C	079	Derivative Spectroscopy Chemiluminigenic Probing of Granulocyte Function in Healthy Controls and Burn Patients	383
		3A161101A91C	081	Cultured Keratinocytes as Epithelial Grafts for Burned Soldiers	402
3A161101A91C	089	3M161102BS10	305	Inequality of VA/Q Ratios Following Smoke Inhalation Injury and the Effect of Angiotensin Analogues	403

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DAOA6380	86 10 01	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62272A	3S162772A874	AD	164
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP	FY87-01		

11. TITLE *(Precede with Security Classification Code)*
(U) Clinical Operation, Center for Treatment of Burned Soldiers

12. SUBJECT AREAS
06 05 Clinical Medicine 06 13 Microbiology 06 15 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
50 07	CONT	DA	C

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED APPROVED BY Paul A Pruitt

a. DATE EFFECTIVE		18. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
c. TYPE	d. AMOUNT	86	53.0	2,006
e. KIND OF AWARD	f. CUM/TOTAL	87	53.0	2,115

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS *(include zip code)* Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR MC MANUS, W F
d. TELEPHONE NUMBER *(include area code)* 512-221-2720	d. TELEPHONE NUMBER *(include area code)* 512-221-3301
21. GENERAL USE FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)* g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Heterograft; (U) Resuscitation; (U) Homograft; (U) Air Evacuation; (U) Thermal Injury; (U) Topical Therapy;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

22. (Continued) (U) Volunteers; (U) Autograft; (U) RAII

23. (U) The Clinical Division of this Institute is the major treatment center for thermally injured military personnel of all services as well as other eligible beneficiaries. The goals of the Division, in addition to the specialized care of severely injured patients, include the investigation of diagnostic and therapeutic technics to improve the survival and function of injured patients as well as promulgation of scientific medical information to health professionals.

24. (U) Thermally injured patients from the Continential United States and throughout the world are transported to this Institute for intensive, specialized treatment. Carefully controlled evaluation of new treatment technics is conducted by the professional staff.

25. (U) 8501 - 8512. One hundred ninety-seven seriously burned patients were admitted and treated at this Institute during calendar year 1985. Current clinical research activities include host resistance studies, endocrine changes following injury, development of optimal nutritional support of the burned patient, the use of skin substitutes, and studies in the control of postinjury infection.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 January 1985 - 31 December 1985

INVESTIGATORS

William F. McManus, MD, Colonel, MC
Anton J. Jirka, MD, Colonel, MC
Kevin J. Farrell, MD, Lieutenant Colonel, MC
Khan Z. Shirani, MD, Lieutenant Colonel, MC
George M. Vaughan, MD, Lieutenant Colonel, MC
Donna W. Kyzar, RN, Lieutenant Colonel, AN
Robert C. Allen, MD, Major, MC
Lawrence W. Lehrner, MD, Major, MC
James C. McKay, MD, Major, MC
Anne E. Missavage, MD, Major, MC
Steven I. Schmidt, MD, Major, MC
Anthony A. Smith, MD, Major, MC
Patricia S. Latona, RN, Major, AN
David W. Mozingo, MD, Captain, MC
L. Willis Roberts, MD, Captain, MC
Gary R. Culbertson, MD, Captain, MC
Ralph D. Cook, MD, Captain, MC
Rosendo T. Gutierrez, Captain, SP
Stephen H. Luster, Captain, SP
Kathleen F. McCoy, Captain, SP
Alvis T. Perry, MD, Captain, MC
Kathryn E. Robertson, RN, Captain, AN
Richard G. Simmons, Jr., Captain, SP
Susan W. Wilson, Captain, SP
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Jan 85 through 31 Dec 85

INVESTIGATORS: William F. McManus, MD, Colonel, MC
Anton J. Jirka, MD, Colonel, MC
Kevin J. Farrell, MD, Lieutenant Colonel, MC
Khan Z. Shirani, MD, Lieutenant Colonel, MC
George M. Vaughan, MD, Lieutenant Colonel, MC
Donna W. Kyzar, RN, Lieutenant Colonel, AN
Robert C. Allen, MD, Major, MC
Lawrence W. Lehrner, MD, Major, MC
James C. McKay, MD, Major, MC
Anne E. Missavage, MD, Major, MC
Steven I. Schmidt, MD, Major, MC
Anthony A. Smith, MD, Major, MC
Patricia S. Latona, RN, Major, AN
David W. Mozingo, MD, Captain, MC
L. Willis Roberts, MD, Captain, MC
Gary R. Culbertson, MD, Captain, MC
Ralph D. Cook, MD, Captain, MC
Rosendo T. Gutierrez, Captain, SP
Stephen H. Luster, Captain, SP
Kathleen F. McCoy, Captain, SP
Alvis T. Perry, MD, Captain, MC
Kathryn E. Robertson, RN, Captain, AN
Richard G. Simmons, Jr., Captain, SP
Susan W. Wilson, Captain, SP
Basil A. Pruitt, Jr., MD, Colonel, MC

One hundred ninety-seven patients were admitted to this Institute during calendar year 1985. Principal activities included care of severely burned patients, research to improve survival and function of such patients, and education and training of health care professionals and paraprofessionals. The areas of research included an analysis of the adaptation of renal function following burn injury, a review of inhalation injury, a study to evaluate the effectiveness and safety of Artificial Skin in the tr atment of third degree flame or scald injuries, a comparative study of E-Z Derm™ and Biobrane®, an ongoing study of five-percent aqueous mafenide acetate soaks for the topical treatment of burn wounds following grafting,

studies of neuroendocrine abnormalities in burn injuries, assessment of thyroid hormone kinetics in thermally injured patients, a multicenter open study of the efficacy, safety, and tolerance of PRIMAXIN® in the parenteral therapy of infections caused by pathogenic bacteria, determination of the pharmacokinetics of amikacin in burned patients, evaluations of IgG and T_4 therapy in burn patients, evaluation of means to reduce blood loss during tangential excision, studies of metabolism and nutritional effects of burn injury in soldiers, and a project to characterize certain biochemical indicators of infection in the thermally injured.

AEROMEDICAL TRANSFER
BIOLOGIC DRESSINGS
EXCISION
IMMUNOMODULATION
INHALATION INJURY
METABOLISM
NEUROENDOCRINE ABNORMALITIES
NUTRITION
PRIMAXIN®
RENAL FUNCTION
RESUSCITATION
SYNTHETIC SKIN SUBSTITUTES
THERMAL INJURY
TOPICAL THERAPY
VASOACTIVE HORMONES

CLINICAL OPERATION
CENTER FOR TREATMENT OF BURNED SOLDIERS

INTRODUCTION

During calendar year 1985, 197 patients were admitted to this Institute and there were 197 patient dispositions. Statistical data are based on the 197 patient dispositions during calendar year 1985. There were 166 males and 31 females with an average age of 30.7, ranging from six months to 90 years of age. Forty patients were less than 16 years old and 40 patients (20.3 percent) were more than 45 years old. The average total body surface area burn of the entire population was 29 percent of the total body surface area with 15.1 percent the average extent of full-thickness injury. The average hospital stay of all patients, excluding convalescent leave for active duty military patients, was 42.6 days. One hundred and forty-six patients (74.1 percent) were admitted within 48 hours of injury.

During calendar year 1985, 700 operative procedures were performed on 128 patients, an average of 5.5 operative procedures per patient. Three hundred and eighty anesthetics were given to 128 patients for an average of 2.9 anesthetics per patient. One hundred and seventeen patients received a total of 833,335 milliliters of blood for an average of 7,122.5 milliliters of blood per patient.

ADMISSIONS DATA

The Clinical Division of this Institute admitted 197 soldiers and other authorized patients with thermal, chemical, or electrical injury during calendar year 1985. Aeromedical teams from the Institute conducted 86 missions within the Continental United States to transfer 98 of the 197 patients (49.7 percent) admitted. Twenty-two missions were carried out by rotary wing aircraft (25.6 percent) and 64 by fixed-wing aircraft (74.4 percent). One hundred twenty-three of the 197 patients (62.4 percent) were admitted within 24 hours of injury and 146 (74.1 percent) were admitted within 48 hours of injury. One hundred fifty-nine patients were male and 38 were female.

DISPOSITIONS DATA

The following statistics are based on 197 patient dispositions during calendar year 1985. The ages of these 197 patients ranged from six months to 90 years with an average age of 30.7 years. Burn sizes averaged 29 percent of the total body surface area with an average full-thickness component of 15.1 percent. Forty patients were in the pediatric age group (age 15 and under) with an average age of 3.8 years and an

average burn size of 15.6 percent of the total body surface area. The average hospital stay of all dispositions was 43.4 days when convalescent leave was included in the calculation and 42.6 days when convalescent leave was excluded. There were eight patients with high voltage electrical injury and four patients with chemical injury. The sources of admission are identified in Table 1 and the causes of burn injury are detailed in Table 2.

Five patients required hemodialysis for acute renal failure. Acute myocardial infarctions were seen in four patients and acute pulmonary emboli in four patients. Inhalation injury was identified in 76 patients (38.6 percent of admissions). Ninety-seven patients (49.2 percent) had some associated injury (includes 76 patients with inhalation injury) which included fractures or dislocations in 12 patients and lacerations in nine patients.

Morbidity and Mortality. Forty-two of the 197 dispositions (21.3 percent) died during calendar year 1985. Autopsies were performed in 26 (61.9 percent) of these hospital deaths. The average burn size of patients who died was 37.6 percent and the full-thickness average was 37.6 percent. Age ranged from 11 months to 90 years of age. Twenty-six of these patients (61.9 percent) had inhalation injury as a primary or contributing cause of death. Three patients died with acute myocardial infarctions, one with acute phenol intoxication, and one with staphylococcal scalded skin syndrome. Four of the forty-two deaths (9.5 percent) occurred in pediatric patients. These children had an average total body surface area burn of 39.7 percent and an average full-thickness burn of 21.4 percent. The average age of children who died was 18.25 months (11 through 23 months). One child had an autopsy.

Infection was once again the most common complication following thermal injury with bacterial pneumonia occurring in 35 patients. The most common organisms isolated in patients with bacterial pneumonia were Staphyloccocus aureus in 24 patients, Escherichia coli in eight patients, and Klebsiella and Pseudomonas species in five patients each. However, only six patients demonstrated bacterial septicemia and no patients had bacterial invasion of the burn wound identified during this calendar year. Two patients had clinical upper gastrointestinal hemorrhage and all responded to noninvasive therapy.

Table 3 lists the effect of age and extent of injury on survival and Table 4 lists mortality rates associated with increments of ten percent of the total body surface area for the years 1979 through 1985. Table 5 summarizes the survival of patients with extensive burns from 1958 through 1985. Table 6 compares mortality before and after the use of topical

TABLE 1

SOURCES OF ADMISSION (1985)*

AREA	A	AD	AF	AFD	N	ND	M	MD	VAB	OTHER	TOTAL
First Army	5	0	1	2	0	2	2	0	0	1	3
Third Army	1	0	1	0	2	0	1	1	5	10	21
Fifth Army	12	12	8	5	0	0	2	1	10	87	137
Sixth Army	9	0	0	0	0	0	3	0	0	2	14
Alaska	0	1	0	0	0	0	0	0	0	0	1
Germany	1	1	0	0	0	0	0	0	0	0	2
Greece	0	0	1	0	0	0	0	0	0	0	1
Hawaii	0	1	0	0	0	0	0	0	0	0	1
Honduras	1	0	0	0	0	0	0	0	0	0	1
Italy	0	0	1	0	0	0	0	0	0	0	1
Japan	1	0	0	0	0	0	0	0	0	0	1
Korea	1	2	0	0	0	0	0	0	0	0	3
Mexico	0	0	1	0	0	0	0	0	0	0	1
TOTAL	31	17	12	7	2	2	8	2	16	100	197

*A = Army, AF = Air Force, N = Navy, M = Marine Corps, D = Dependent, VAB = Veterans Administration Beneficiary, and OTHER = Civilian Emergency, US Public Health Service Beneficiary, and Bureau of Employees Compensation Beneficiary

TABLE 2

BURN ETIOLOGY (1985)

Causes	Number of Patients	Disposition (Percent)	Deaths	Mortality (Percent)
Gasoline, Diesel, and Kerosene	54	27.4	4	9.5
Hot Liquids	27	13.7	4	9.5
Structural Fires	25	12.7	11	26.2
Butane, Propane, or Natural/Sewer Gas Explosions	23	11.7	11	26.2
Motor Vehicle Accidents	22	11.2	4	9.5
Electrical	10	5.1	1	2.4
Contact	8	4.1	1	2.4
Bomb, Shell, Simulator Grenade, and Gunpowder Explosions	7	3.6	-	-
Other	7	3.6	-	-
Open Flames	6	3.0	4	9.5
Chemical	4	2.0	1	2.4
Welding	3	1.5	-	-
Aircraft Accidents	1	0.5	1	2.4
TOTAL	197		42	

TABLE 3

AGE, BODY SURFACE INVOLVEMENT, AND MORTALITY (1985)

Age (Years)	Percent Total Body Surface Area Burn										Total Cases	Total Deaths	Mortality (Percent)
	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100			
0 - 1	2	3	2	-	1	-	-	-	-	-	8	1	12.5
1 - 2	4	2	2(1)	1(1)	1(1)	-	-	-	-	-	10	3	30.0
2 - 3	2	2	-	-	1	-	-	-	-	-	5	-	-
3 - 4	3	1	-	1	-	-	-	-	-	-	5	-	-
4 - 5	1	1	1	-	-	-	-	-	-	-	3	-	-
5 - 10	2	-	-	1	-	-	-	-	-	-	3	-	-
10 - 15	1	1	-	2	-	-	-	-	-	-	4	-	-
15 - 20	4	2	1	-	-	2	-	-	-	-	9	-	-
20 - 30	10	10	8	16(1)	7(1)	3	2	4(4)	1(1)	1(1)	63	8	14.3
30 - 40	5	8	5	4	3	2(1)	2	1(1)	3(3)	1(1)	33	6	15.2
40 - 50	2	5	2	-	6(4)	-	2(2)	1	-	-	18	6	33.3
50 - 60	2(1)	3	2(1)	1	1(1)	3(1)	2(2)	-	-	1(1)	15	7	46.7
60 - 70	2(1)	2	3(1)	1	-	-	-	-	-	1(1)	9	3	33.3
70 - 80	1	2	1(1)	1(1)	-	-	-	-	-	-	5	2	40.0
80 - 90	-	4(3)	1(1)	-	-	-	-	-	1(1)	-	6	5	83.3
90 - 100	-	-	-	-	-	-	1(1)	-	-	-	1	1	100.0
Total Cases	41(2)	46(3)	28(5)	28(3)	19(7)	11(3)	9(5)	6(5)	5(5)	4(4)	197		
Total Deaths	2	3	5	3	7	3	5	5	5	4		42	
Mortality (Percent)	4.9	6.5	17.9	10.7	36.8	27.3	55.6	83.3	100.0	100.0			21.3

TABLE 4

PERCENT BODY SURFACE BURN INVOLVEMENT AND MORTALITY (1982 - 1985)

	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
1985											
Number of Patients	41	46	28	28	19	11	9	6	5	4	197
Number of Deaths	2	3	5	3	7	3	5	5	5	4	42
PERCENT MORTALITY	4.9	6.5	17.9	10.7	36.8	27.3	55.6	83.3	100.0	100.0	21.3
1984											
Number of Patients	46	38	31	23	18	13	7	5	6	3	190
Number of Deaths	-	-	2	8	4	6	2	3	6	3	34
PERCENT MORTALITY	-	-	6.5	34.8	22.2	46.2	28.6	60.0	100.0	100.0	17.9
1983											
Number of Patients	47	31	30	30	13	8	6	4	5	5	179
Number of Deaths	1	3	6	7	3	3	5	3	4	5	40
PERCENT MORTALITY	2.1	9.7	20.0	23.3	23.0	37.5	83.3	75.0	80.0	100.0	22.3
1982											
Number of Patients	42	51	39	29	25	17	12	7	7	2	231
Number of Deaths	-	3	5	6	11	6	8	6	7	2	54
PERCENT MORTALITY	-	5.9	12.8	20.7	44.0	35.3	66.7	85.7	100.0	100.0	23.4

TABLE 5

SURVIVAL AND NONSURVIVAL BY YEAR FOR PATIENTS WITH EXTENSIVE BURNS (OVER 30 PERCENT OF TOTAL BODY SURFACE AREA) FROM 1962 TO 1985

Year	SURVIVORS			NONSURVIVORS		
	Number of Cases	Average Percent Burn		Number of Cases	Average Percent Burn	
		Total	Third Degree		Total	Third Degree
1985	48	43.6	21.7	42	54.3	37.1
1984	43	46.4	24.8	32	59.5	38.7
1983	37	43.5	17.5	30	62.8	50.7
1982	53	43.7	24.8	54	53.9	38.3
1981	54	42.7	17.5	43	62.2	39.8
1980	62	42.7	15.1	66	64.3	41.8
1979	61	45.4	13.4	74	65.0	37.0
1978	67	45.7	14.8	69	55.2	33.0
1977	66	42.2	14.4	70	56.9	29.0
1976	69	45.5	15.0	79	64.2	31.1
1975	80	46.1	14.7	94	61.3	32.8
1974	55	43.9	12.2	97	60.8	35.9
1973	47	43.7	19.6	113	60.3	36.2
1972	62	42.0	17.2	103	56.7	35.9
1971	63	41.9	14.0	68	60.8	38.0
1970	92	39.4	10.7	70	51.9	32.6
1969	113	43.2	11.1	70	58.7	26.4
1968	143	44.2	12.6	38	54.6	24.6
1967	103	42.7	13.3	51	59.9	32.3
1966	68	41.5	14.9	59	59.9	31.3
1965	47	43.8	21.0	33	66.0	33.4
1964	40	41.8	14.8	37	65.0	42.4
1963	28	45.8	19.6	57	69.0	41.0
1962	18	42.7	21.4	54	59.1	46.2

TABLE 6

COMPARISON OF BURN MORTALITY RATES (1962 - 1963 AND 1964 - 1985)

	PERCENT TOTAL BODY SURFACE AREA BURN														
	0-30			30-40			40-50			50-60			60-100		
	Number of Patients	Number of Deaths	Mortality (Percent)	Number of Patients	Number of Deaths	Mortality (Percent)	Number of Patients	Number of Deaths	Mortality (Percent)	Number of Patients	Number of Deaths	Mortality (Percent)	Number of Patients	Number of Deaths	Mortality (Percent)
1962 - 1963	140	6	4.3	36	16	44.4	36	22	61.1	23	18	78.3	55	49	89.1
1964 - 1985	2,886	109	3.8	817	153	8.7	648	207	31.9	467	225	48.2	843	710	84.2
1985	115	10	8.7	28	3	10.7	19	7	36.8	11	3	27.3	24	19	79.2

chemotherapy of the burn wound. Table 7 lists the causes of death for calendar year 1985.

EDUCATIONAL ACTIVITIES

During calendar year 1985, the professional staff of the Clinical Division continued to provide education to all professional and paraprofessional groups, at the local, national, and international levels. A total of 23 resident physicians were attached for periods of one to three months, including three each from Letterman Army Medical Center, Wilford Hall Medical Center, and William Beaumont Hospital (Royal Oak, Michigan), two each from Brooke Army Medical Center, Fitzsimons Army Medical Center, Travis Air Force Base Medical Center, and the Medical College of Wisconsin, and one each from the Naval Aerospace Medical Institute, William Beaumont Army Medical Center, Kettering Medical Center (Dayton, Ohio), Suburban General Hospital (Norristown, Pennsylvania), the University of Texas Health Science Center at San Antonio (Texas), Spartansburg South Carolina Medical System. A total of 12 medical students, including four health profession scholarship medical students, rotated, which included two students from Loyola University in Chicago and one student each from the University of Virginia, the University of Colorado, Columbia University in New York City, Louisiana State University, the University of Texas Health Science Center at San Antonio (Texas), and the Southeast Osteopathic Medical College in Miami. A total of 16 physicians visited from foreign countries for periods ranging from one day to one year which included four from the Dominican Republic, three from Germany, two from Japan, and one each from China, Pakistan, Norway, Jordan, Great Britain, Canada, and the Ivory Coast. The Respiratory Therapy Branch had 150 trainees, the Physical Therapy Branch had 36 trainees, and the Occupational Therapy Branch had nine trainees. Twenty-two scientific publications appeared in referenced medical journals and approximately 130 scientific presentations were conducted for military and civilian audiences. Numerous scientific presentations were made at the Academy of Health Sciences and various military installations throughout the continential United States, to include support of the Battlefield Medicine Course for the United States Air Force and the Combat Casualty Courses for the United States Army. In addition, weekly professional staff conferences were conducted for and by Institute personnel.

TABLE 7

CAUSES OF DEATH (1985)

Patient	Age	Sex	PERCENT BURN Total	PERCENT BURN Third Degree	Postburn Day	Cause of Death
1	34	M	90	77	12	90 percent total body surface area burn with inhalation injury and acute myocardial infarction.
2	23	M	71	71	2	71 percent total body surface area burn with inhalation injury and acute phenol intoxication.
3	81	F	20	18	26	20 percent total body surface area burn with bronchopneumonia.
4	83	F	85	75	5	85 percent total body surface area burn with acute renal failure and inhalation injury.
5	54	M	68	22	3	*68 percent total body surface area burn with arteriosclerotic heart disease and inhalation injury.
6	75	M	38	31	2	38 percent total body surface area burn with inhalation injury and bronchopneumonia.
7	49	M	46	35	27	46 percent total body surface area burn with inhalation injury and bronchopneumonia.
8	40	M	69	62	48	*69 percent total body surface area burn with inhalation injury and multiorgan system failure (respiratory, hepatic, renal).
9	88	F	17	-	8	17 percent total body surface area burn with inhalation injury and secondary bronchopneumonia.

*Autopsy Not Performed

Patient	Age	Sex	PERCENT BURN Total	PERCENT BURN Third Degree	Postburn Day	Cause of Death
10	27	M	73	39	13	73 percent total body surface area burn with severe inhalation injury and pneumonia.
11	53	M	97	97	1	97 percent total body surface area burn with severe inhalation injury.
12	43	F	46	38	36	*46 percent total body surface area burn with invasive burn wound infection with Aspergillus and Mucor species.
13	29	M	79	67	80	79 percent total body surface area burn with bronchopneumonia.
14	73	M	22	18	129	*22 percent total body surface area burn with inhalation injury and cerebral hypoxia.
15	1	M	22	2	7	*22 percent total body surface area burn with inhalation injury and cerebral hypoxia.
16	60	M	94	84	41	94 percent total body surface area burn with inhalation injury and pneumonia.
17	20	M	34	7	40	34 percent total body surface area burn with inhalation injury and pneumonia.
18	84	F	23	22	55	23 percent total body surface area burn with arteriosclerotic heart disease.
19	1	M	49	49	6	*49 percent total body surface area burn with acute cerebral edema.
20	87	M	16	2	78	16 percent total body surface area burn with perforated sigmoid diverticulum with abscess and sepsis.

*Autopsy Not Performed

Patient	Age	Sex	PERCENT BURN		Postburn Day	Cause of Death
			Total	Third Degree		
21	55	M	59	25	24	59 percent total body surface area burn with inhalation injury, herpes simplex, and pneumonia.
22	30	M	82	30	8	82 percent total body surface area burn with inhalation injury and pneumonia.
23	63	F	29	24	22	*29 percent total body surface area burn with pneumonia and congestive heart failure.
24	23	M	90	84	9	*90 percent total body surface area burn with inhalation injury and pneumonia.
25	27	M	84	76	14	84 percent total body surface area burn with inhalation injury and pneumonia.
26	1	M	38	16	33	38 percent total body surface area burn with staphylococcal and scalded skin syndrome.
27	41	M	45	37	52	*45 percent total body surface area burn with inhalation injury and pneumonia.
28	54	M	9	7	26	8.5 percent total body surface area burn with alcoholic cardiomyopathy and Laennec's cirrhosis.
29	61	M	6	5	4	6 percent total body surface area burn with aspiration and arrest.
30	33	M	56	2	24	*56 percent total body surface area burn with inhalation injury and bronchopneumonia.
31	0	M	50	19	29	*50 percent total body surface area burn with abdominal (lesser sac) abscess secondary to perforated gastric ulcer.

*Autopsy Not Performed

Patient	Age	Sex	PERCENT BURN		Postburn Day	Cause of Death
			Total	Third Degree		
32	44	M	61	8	37	61 percent total body surface area burn with inhalation injury and pneumonia.
33	46	F	48	42	9	*48 percent total body surface area burn with inhalation injury and pneumonia.
34	38	M	70	8	21	70 percent total body surface area burn with cardiomyopathy, inhalation injury, pneumonia, and associated fungal burn wound infection.
35	31	M	87	79	55	*87 percent total body surface area burn with inhalation and bronchopneumonia.
36	57	M	28	26	7	*28 percent total body surface area burn with inhalation injury, pneumonia, and chronic alcoholism.
37	59	F	50	48	34	50 percent total body surface area burn with acute myocardial infarction, inhalation, and pneumonia.
38	20	M	47	35	16	47 percent total body surface area burn with inhalation injury and pneumonia.
39	28	M	74	10	26	74 percent total body suface area burn with necrotizing enterocolitis.
40	90	M	69	67	11	69 percent total body surface area burn with inhalation injury and bronchopneumonia.
41	35	M	81	80	18	*81 percent total body surface area burn with inhalation injury and bronchopneumonia.
42	58	M	70	42	13	70 percent total body surface area burn with bronchopneumonia.

*Autopsy Not Performed

PRESENTATIONS

Robertson KE: Overview of nursing at the US Army Institute of Surgical Research. Presented to the Clinical Specialist Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 January 1985.

Dimmick D: Overview of nursing at the US Army Institute of Surgical Research. Presented to the Clinical Specialist Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 January 1985.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infection in severely injured patients. Presented to the Department of Surgery, University of Alabama, Birmingham, Alabama, 17-19 January 1985.

Pruitt BA Jr: Fluid resuscitation. Presented to the Department of Surgery, University of Alabama, Birmingham, Alabama, 17-19 January 1985.

Roberts LW: Burns. Presented to the Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 18 January 1985.

Robertson KE: Emergency management and transport of burn patients. Presented to the Aviators Course (2CF7), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 18 January 1985.

Pruitt BA Jr: Epidemiology, triage, and pathophysiology of thermal injuries. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Fort Sam Houston, San Antonio, Texas, 21 January 1985.

Cozean RJ: Psychosocial response to thermal injury. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Fort Sam Houston, San Antonio, Texas, 21-28 January 1985.

Crawford J: Grafts, donors, and dressings. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Fort Sam Houston, San Antonio, Texas, 21-28 January 1985.

Hollan E: Infection control. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Fort Sam Houston, San Antonio, Texas, 21-28 January 1985.

McManus WF: Management of the burn wound. Presented at the OT/PT Conference on Management of Burns in the Theater of

Operations, Fort Sam Houston, San Antonio, Texas, 21-28 January 1985.

McManus WF: Pulmonary complications. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Fort Sam Houston, San Antonio, Texas, 21-28 January 1985.

McManus WF: Thermal, electrical, and chemical injuries. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Fort Sam Houston, San Antonio, Texas, 21-28 January 1985.

Robertson KE: Thermal, electrical, and chemical injuries and field triage and management in the combat zone. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Fort Sam Houston, San Antonio, Texas, 21-28 January 1985.

Pruitt BA Jr: Metabolic response to trauma. Presented to the Department of Surgery Literature Conference, University of Texas Medical School, San Antonio, Texas, 30 January 1985.

Robertson KE: Emergency management, wound care, and complications of burns. Presented to the Critical Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 30 January 1985.

Wilson SW: Nutritional management of the burn patient. Presented to the Dietetic Interns, Baptist Memorial Hospital, San Antonio, Texas, 12 February 1985.

McManus WF: Infections in burns. Presented at the Infections in Burns Seminar, Dallas, Texas, 15 February 1985.

Pruitt BA Jr: Infection control in burn patients. Presented at the American Pharmaceutical Association Meeting, San Antonio, Texas, 17 February 1985.

McManus WF: Role of fluids and pharmacologic agents in burn patient resuscitation. Presented to the Federal Section of the American Pharmaceutical Association Meeting, San Antonio, Texas, 19 February 1985.

Pruitt BA Jr: The diagnosis and treatment of opportunistic infections in injured patients. Presented to the Department of Surgery, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, 19-21 February 1985.

Pruitt BA Jr: Opportunistic infections in the severely injured patient. Presented at the Second International

Symposium on the Pathophysiology of Combined Injury and Trauma, Wintergreen, Virginia, 26-28 February 1985.

Pruitt BA Jr: Immunomodulators in Burn Management. Presented at the IGIV Workshop, New Orleans, Louisiana, 2 March 1985.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections and Gram-negative sepsis in stressed patients. Presented to the Department of Surgery, Louisiana State University, Shreveport, Louisiana, 4-5 March 1985.

Pruitt BA Jr: Metabolic response and nutritional support in patients with major trauma. Presented to the Department of Surgery, Louisiana State University, Shreveport, Louisiana, 4-5 March 1985.

Kyzar DW: An overview of the Nursing Service Branch at the US Army Institute of Surgical Research. Presented to the Nurse Educator Tour, US Army Recruiting Command, Fort Sam Houston, San Antonio, Texas, 6 March 1985.

Wilson SW: Nutritional management of the burn patient. Presented to the dietitians, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 6 March 1985.

Pruitt BA Jr: Infection and burn problems. Presented to the Combat Casualty Management Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 March 1985.

Robertson KE: Initial management of burns. Presented to the Aviator Course (2CF7), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 15 March 1985.

Roberts LW: Burns. Presented to the Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 21 March 1985.

Pruitt BA Jr: Infection and burn problems. Presented to the Combat Casualty Management Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 25 March 1985.

Pruitt BA Jr: Burn care and research in the United States Army. Presented at the Fifth Annual Meeting of the Uniformed Services University Surgical Associates, Uniformed Services University of the Health Sciences, Bethesda, Maryland, 5 April 1985.

Robertson KE: Emergency management of burns. Presented to the Northeast Volunteer Fire Department, San Antonio, Texas, 8 April 1985.

McCoy KF: Care of the thermally injured patient. Presented to the Physical Therapy Specialist Class (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 April 1985.

Robertson KE: Overview of burn care. Presented at the Nursing Education and Training Department Symposium, Critical Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 26 April 1985.

Robertson KE: Overview of burn care. Presented to the US Army Hospital Reserve Unit, Fort Sam Houston, San Antonio, Texas, 26 April 1985.

McManus WF: Mass casualty care: triage of burn patients. Presented at the Medical Symposium, 2291st US Army Hospital and 112th Medical Brigade, Columbus, Ohio, 27 April 1985.

Robertson KE: Air transfer of burn and combat-injured patients. Presented at the Medical Symposium, 2291st US Army Hospital and 112th Medical Brigade, Columbus, Ohio, 27 April 1985.

Robertson KE: Triage: a multidimensional approach. Presented at the Medical Symposium, 2291st US Army Hospital and 112th Medical Brigade, Columbus, Ohio, 27 April 1985.

Shirani KZ: Effects of environment on infection in burn patients. Presented at the Fifth Annual Meeting of the Surgical Infection Society, New Orleans, Louisiana, 29 April 1985.

Pruitt BA Jr: Host-opportunistic interactions in surgical infection. Presented at the Fifth Annual Meeting of the Surgical Infection Society, New Orleans, Louisiana, 30 April 1985.

Luster SH: Occupational therapy in burn rehabilitation. Presented to the Occupational Therapy Assistant Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 1 May 1985.

Cozean RJ: Patient and family perspectives of the burn experience. Presented at the US Army Institute of Surgical Research Nursing Service Branch Burn Symposium, Fort Sam Houston, San Antonio, Texas, 8-9 May 1985.

Cozean RJ: Psychological focus in burn management. Presented at the US Army Institute of Surgical Research Nursing Service Branch Burn Symposium, Fort Sam Houston, San Antonio, Texas, 8-9 May 1985.

Hidenrite D: Burn wound care. Presented at the US Army Institute of Surgical Research Nursing Service Branch Burn Symposium, Fort Sam Houston, San Antonio, Texas, 8-9 May 1985.

Hollan E: Infection control in the burn unit. Presented at the US Army Institute of Surgical Research Nursing Service Branch Burn Symposium, Fort Sam Houston, San Antonio, Texas, 8-9 May 1985.

Kyzar DW: A historical perspective of burn management. Presented at the US Army Institute of Surgical Research Nursing Service Branch Burn Symposium, Fort Sam Houston, San Antonio, Texas, 8-9 May 1985.

McManus WF: Inhalation injury and pulmonary complications. Presented at the US Army Institute of Surgical Research Nursing Service Branch Burn Symposium, Fort Sam Houston, San Antonio, Texas, 8-9 May 1985.

O'Grady S: Hemodynamic monitoring of the burn-injured patient. Presented at the US Army Institute of Surgical Research Nursing Service Branch Burn Symposium, Fort Sam Houston, San Antonio, Texas, 8-9 May 1985.

Pruitt BA Jr: Diagnosis and treatment of burn wound infection. Presented at the US Army Institute of Surgical Research Nursing Service Branch Burn Symposium, Fort Sam Houston, San Antonio, Texas, 8-9 May 1985.

Robertson KE: Thermal, chemical, and electrical injury - emergency management. Presented at the US Army Institute of Surgical Research Nursing Service Branch Burn Symposium, Fort Sam Houston, San Antonio, Texas, 8-9 May 1985.

Worden S: The multidisciplinary approach to burn care. Presented at the US Army Institute of Surgical Research Nursing Service Branch Burn Symposium, Fort Sam Houston, San Antonio, Texas, 8-9 May 1985.

Zellers L: Aeromedical transfer of burn-injured patients. Presented at the US Army Institute of Surgical Research Nursing Service Branch Burn Symposium, Fort Sam Houston, San Antonio, Texas, 8-9 May 1985.

McManus WF: Treatment of burns. Presented to the Scientific Assembly, Texas Medical Association, San Antonio, Texas, 9 May 1985.

Simons RG: Physical therapy in burn care. Presented at the US Army Institute of Surgical Research Nursing Service Branch Symposium, San Antonio, Texas, 9 May 1985.

Pruitt BA Jr: Update on burns. Presented to the Texas Society of Plastic Surgeons, San Antonio, Texas, 11 May 1985.

Everson L: Burns, intraoperative care. Presented to the Army Nurse Corps Operating Room Nurse Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 16 May 1985.

Kyzar DW: Overview of the Nursing Service Branch at the US Army Institute of Surgical Research. Presented to the Nurse Educator Tour, US Army Recruiting Command, Fort Sam Houston, San Antonio, Texas, 20 May 1985.

McManus WF: Initial management of the burn patient. Presented to the Emergency Medicine Residents, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 23 May 1985.

Pruitt BA Jr: The metabolic changes and nutritional support of severely injured patients. Presented at the Charlotte Memorial Hospital and Medical Center, Charlotte, North Carolina, 23 May 1985.

Robertson KE: Emergency management and wound car of burns. Presented at the Wilford Hall Medical Center, Lackland Air Force Base, San Antonio, Texas, 3 June 1985.

Pruitt BA Jr: Current treatment of severe burns. Presented to the Association of Surgical Technologists, Inc., San Antonio, Texas, 12 June 1985.

Kyzar DW: Overview of the Chief Nurse's role, US Army Institute of Surgical Research. Presented to the Army Medical Department Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 13 June 1985.

Robertson KE: Overview of burn management. Presented to the Physician's Assistant Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 19 June 1985.

Robertson KE: Overview of burn management. Presented to the Physical Therapy Course, Academy of Health Sciences, Fort Sam Houston, Texas, 19 June 1985.

Robertson KE: Overview of burn management. Presented to the Graduate Nursing Students, University of Texas Health Science Center, San Antonio, Texas, 21 June 1985.

Pruitt BA Jr: Care and closure of the burn wound. Presented at the Mount Sinai Surgical Grand Rounds, Miami Beach, Florida, 24 June 1985.

Roberts LW: Burns. Presented to the Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 24 and 26 June 1985.

Pruitt BA Jr: Diagnosis, treatment, and prevention of overwhelming infection: the role of IGIV. Presented to the Plastic and Reconstructive Division, Saint Francis Memorial Hospital, San Francisco, California, 27-28 June 1985.

Pruitt BA Jr: Hemodynamic changes and fluid resuscitation of severely burned patients. Presented to the Department of Medicine, Oregon Health Sciences University, Portland, Oregon, 1-2 July 1985.

Pruitt BA Jr: The diagnosis and treatment of opportunistic infections in injured man. Presented at the Olin E. Teague Veteran's Center, Temple, Texas, 16 July 1985.

Pruitt BA Jr: Surgical management of burns. Presented at the Scott and White Clinic, Temple, Texas, 16 July 1985.

Roberts LW: Burns. Presented to the Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 17 and 22 July 1985.

Jordan BS: Management of the burn patient. Presented to the Critical Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 2 August 1985.

Hildenrite D: Management of the burn patient. Presented to the Critical Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 2 August 1985.

Cozean RJ: Management of the burn patient - psychosocial considerations. Presented to the Critical Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 2 August 1985.

Latona PS: An overview of the Nursing Service Branch at the US Army Institute of Surgical Research. Presented to the Nurse Educator Tour, US Army Recruiting Command, Fort Sam Houston, San Antonio, Texas, 14 August 1985.

McCoy KF: Care of the thermally injured patient. Presented to Physical Therapy Specialist Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 14 August 1985.

Shippe RL: Homeostatic control of zinc in the burned rat. Presented to the Mary Lipscomb Hamrick Army Medical Specialist Corps Research Course, Walter Reed Army Medical Center, Washington, DC, 16 August 1985.

Wilson SW: Trace mineral supplementation of burn patients - a national survey. Presented to the Mary Lipscomb Hamrick Army Medical Specialist Corps Research Course, Walter Reed Army Medical Center, Washington, DC, 16 August 1985.

O'Grady S: Blood products and their administration. Presented to the Critical Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 6 September 1985.

Pruitt BA Jr: Injured-induced alterations of neurohormonal regulation. Presented to the International Surgical Group, Basel, Switzerland, 11 September 1985.

Roberts LW: Burns. Presented to the Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 18 September 1985.

Pruitt BA Jr: The diagnosis and treatment of infections in burn patients. Presented at the Surgical Infection Symposium, San Francisco General Hospital, San Francisco, California, 19-21 September 1985.

Cozean RJ: Psychosocial needs of burn patients and their families. Presented to the Chaplain Residents, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 24 September 1985.

Pruitt BA Jr: Management of the pulmonary injury. Presented at the First National Conference on Pediatric Trauma, New England Medical Center, Boston, Massachusetts, 26-27 September 1985.

Pruitt BA Jr: Metabolic management and nutritional support. Presented at the First National Conference on Pediatric Trauma, New England Medical Center, Boston, Massachusetts, 26-27 September 1985.

Pruitt BA Jr: Diagnosis and treatment of infection in severely injured patients. Presented at the Physicians Forum, IGIV Conference, Houston, Texas, 1 October 1985.

Latona PS: An overview of the Nursing Service Branch at the US Army Institute of Surgical Research. Presented to the Practical Nurse Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 4 October 1985.

Roberts LW: Burns. Presented to the Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 16 October 1985.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in injured man. Presented to the Department of Surgery, University of Vermont, Burlington, Vermont, 24 October 1985.

Pruitt BA Jr: Future concepts of the care of military burn patients. Presented at the Armed Forces Radiobiology Research Institute, Bethesda, Maryland, 25 October 1985.

Cozean RJ: Psychosocial aspects of care and management of the burn patient. Presented at the Third Annual Military Health Professionals Symposium, 2290th US Army Hospital, Rockville, Maryland, 26 October 1985.

Kyzar DW: Comprehensive nursing care for the burn-injured patient. Presented at the Third Annual Military Health Professionals Symposium, 2290th US Army Hospital, Rockville, Maryland, 26 October 1985.

McCoy KF: Role of physical therapy and occupational therapy in burn rehabilitation. Presented at the Third Annual Military Health Professionals Symposium, 2290th US Army Hospital, Rockville, Maryland, 26 October 1985.

McManus WF: Resuscitation and early care of the burn patient. Presented at the Third Annual Military Health Professionals Symposium, 2290th US Army Hospital, Rockville, Maryland, 26 October 1985.

Pruitt BA Jr: Care and closure of the burn wound. Presented at the Third Annual Military Health Professionals Symposium, 2290th US Army Hospital, Rockville, Maryland, 26 October 1985.

Pruitt BA Jr: Epidemiology, first aid triage, and aeromedical transfer of the burn casualty. Presented at the Third Annual Military Health Professionals Symposium, 2290th US Army Hospital, Rockville, Maryland, 26 October 1985.

Gutierrez RT: Care of the thermally injured patient. Presented to the Physical Therapy Specialist Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 30 October 1985.

Zellers LA: Transport of the burn victim. Presented to the Aviator Course (2CF7), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 30 October 1985.

Gutierrez RT: Physical therapy in burns. Presented to the Physical Therapy School students from Southwest Texas State University, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 4 November 1985.

McCoy KF: Physical therapy in burns. Presented to the Physical Therapy School students from Southwest Texas State University, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 4 November 1985.

Pruitt BA Jr: Current treatment of the burn wound. Presented at the Waterbury Hospital, Waterbury, Connecticut, 7 November 1985.

Latona PS: Initial management of the burn victim. Presented to the Reserve Nurses, 94th General Army Reserve Unit, Fort Hood, Texas, 9 November 1985.

Jordan BS: Wound management of the burn victim. Presented to the Reserve Nurses, 94th General Army Reserve Unit, Fort Hood, Texas, 9 November 1985.

Pruitt BA Jr: Planning and design of burn centers. Presented at the Third Annual Harvey A. Beffa Shriners Burn Institute Conference, Galveston, Texas, 10-12 November 1985.

Kyzar DW: An overview of the Nursing Service Branch at the US Army Institute of Surgical Research. Presented to the Nurse Educator Tour, US Army Recruiting Command, Fort Sam Houston, San Antonio, Texas, 13 November 1985.

Luster SH: Rehabilitation of the burn-injured veteran. Presented at the Annual Meeting of the Association of Military Surgeons of the United States, Anaheim, California, 13 November 1985.

Wilson SW: Burn information system - nutrition module. Presented to the Symposium on Computer Applications in Medical Care, Baltimore, Maryland, 13 November 1985.

McManus WF: Electrical burns. Presented at the Partnerships Beyond Survival Seminar, Baptist Medical Center, Oklahoma Medical Center, Oklahoma City, Oklahoma, 14-15 November 1985.

McManus WF: Emergency care, triage, evacuation, and fluid resuscitation. Presented at the Partnerships Beyond Survival Seminar, Baptist Medical Center, Oklahoma Medical Center, Oklahoma City, Oklahoma, 14-15 November 1985.

McManus WF: Infection control in the burn center. Presented at the Partnerships Beyond Survival Seminar, Baptist Medical Center, Oklahoma Medical Center, Oklahoma City, Oklahoma, 14-15 November 1985.

Pruitt BA Jr: Care and coverage of the burn wound. Presented at the Burn Center Symposium, Baptist Medical Center, Oklahoma City, Oklahoma, 14-15 November 1985.

Pruitt BA Jr: Inhalation injury and pulmonary complications. Presented at the Burn Center Symposium, Baptist Medical Center, Oklahoma City, Oklahoma, 14-15 November 1985.

Cozean RJ: Psychosocial aspects of the burn victim. Presented to the Alabama State Nurses Association, Southeast Recruiting Command, 20 November 1985.

Jordan BS: Wound management of the burn victim. Presented to the Alabama State Nurses Association, Southeast Recruiting Command, 20 November 1985.

Latona PS: Initial management of the burn victim. Presented to the Alabama State Nurses Association, Southeast Recruiting Command, 20 November 1985.

Culbertson GR: Burns. Presented to the Special Operations Medical Sergeants Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 2 December 1985.

Latona PS: Initial management of the burn victim. Presented to the Luling Texas Paramedic Association, Luling, Texas, 5 December 1985.

Zellers LA: Initial management of the burn victim. Presented to the Luling Texas Paramedic Association, Luling, Texas, 5 December 1985.

Pruitt BA Jr: Immunologic responses to burn injury. Presented at the Fifth Annual Comprehensive Care of the Burn Patient Conference, Kansas City, Kansas, 6-7 December 1985.

Pruitt BA Jr: Pathophysiology of inhalation injury. Presented at the Fifth Annual Comprehensive Care of the Burn Patient Conference, Kansas City, Kansas, 6-7 December 1985.

Wilson SW: Nutritional management of the burn patient. Presented to Dietetic Students, Incarnate Word College, San Antonio, Texas, 10 December 1985.

Kyzar DW: An overview of the Nursing Service Branch at the US Army Institute of Surgical Research. Presented to the Army Medical Department Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 December 1985.

Latona PS: Burn patient management. Presented to the Aviator Course (2CF7), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 December 1985.

Latona PS: Critical care. Presented to the Short Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 13 December 1985.

PUBLICATIONS

Amy BW, McManus WF, Goodwin CW Jr, and Pruitt BA Jr: Lightning injury with survival in five patients. JAMA 253(2):243-245, January 1985.

Goodwin CW Jr and McManus WF: Viral infections in burned patients. Clin Immunol Today 12:3-9, January 1985.

Pruitt BA Jr, Goodwin CW Jr, Vaughan GM, Aulick LH, Newman JJ, Strome DR, and Mason AD Jr: The metabolic problems of the burn patient. Acta Chir Scand (Suppl) 522:119-139, January 1985.

Robertson KE, Cross PJ, and Terry JC: Burn care: the crucial first days. Am J Nursing 85(1):29-47, January 1985.

McManus AT, McManus WF, Mason AD Jr, Aitcheson AR, and Pruitt BA Jr: Microbial colonization in a new intensive care burn unit. A prospective cohort study. Arch Surg 120(2):217-223, February 1985.

Lund T, Goodwin CW, McManus WF, Shirani KZ, Stallings RJ, Mason AD Jr, and Pruitt BA Jr: Upper airway sequelae in burn patients requiring endotracheal intubation or tracheostomy. Ann Surg 201(3):374-382, March 1985.

Vaughan GM, Mason AD Jr, McManus WF, and Pruitt BA Jr: Alterations of mental status and thyroid hormones after thermal injury. J Clin Endocrinol Metab 60(6):1221-1225, June 1985.

Amy BW, McManus WF, and Pruitt BA Jr: Tetanus following a major thermal injury. J Trauma 25(7):654-655, July 1985.

Cook RD, McManus WF, and Pruitt BA Jr: Management of fractures in burn patients (abstract). J Trauma 25(7):681, July 1985.

Vaughan GM, Becker RA, Unger RH, Ziegler MG, Siler-Khodr TM, Pruitt BA Jr, and Mason AD Jr: Nonthyroidal control of metabolism after burn injury: possible role of glucagon. Metabolism 34(7):637-641, July 1985.

McManus WF: The burn patient: defining the clinical situation. The significance of Pseudomonas in the burn patient. Bull Clin Rev Burn Inj II:22-31, July/August/September 1985.

Yurt RW and Pruitt BA Jr: Decreased wound neutrophils and indiscrete margination in the pathogenesis of wound infection. Surgery 98(2):191-198, August 1985.

Amy BW, McManus WF, Goodwin CW, Mason AD Jr, and Pruitt BA Jr: Thermal injury in the pregnant patient. Surg Gynecol Obstet 161:209-212, September 1985.

Vaughan GM, Shirani KZ, Vaughan MK, Pruitt BA Jr, and Mason AD Jr: Hormonal changes in burned hamsters. Endocrinol 117(3):1090-1095, September 1985.

Kim SH, Hubbard GB, Worley BL, McManus WF, Mason AD Jr, and Pruitt BA Jr: A rapid section technique for burn wound biopsy. JBCR 6(5):433-435, September/October 1985.

Shirani KZ, Vaughan GM, Pruitt BA Jr, and Mason AD Jr: Reduced serum T_4 and T_3 and their altered serum binding after burn injury in rats. J Trauma 25(10):953-958, October 1985.

Kim SH, Hubbard GB, McManus WF, Mason AD Jr, and Pruitt BA Jr: Frozen section technique to evaluate early burn wound biopsy: a comparison with the rapid section technique. J Trauma 25(12):1134-1137, December 1985.

Pruitt BA Jr: The diagnosis and treatment of infection in the burn patient. Burns Incl Therm Inj 11(2):79-91, December 1985.

Burleson DG: Clinical applications of flow cytometry (abstract). Proc Soc Armed Forces Mil Lab Sci 14:17, 1985.

Burleson DG, Vaughan GK, and Mason AD Jr: Differences in the measurement of human neutrophil respiratory burst oxidative activity using molecular probes. Fed Proc (Abstract 989) 44:580, 1985.

Pruitt BA Jr, Mason AD Jr, McManus WF, Bush KW, and Hoffman BE: Burn injury: panel presentation. Trans Assoc Life Insur Med Dir Am 67:158-186, 1985.

Vaughan GM, Mason AD Jr, and Reiter RJ: Serum melatonin after a single aqueous subcutaneous injection in Syrian hamsters. Neuroendocrinol 42:124-127, 1985.

Vaughan GM and Pruitt BA Jr: Pineal-induced depression of free thyroxine in Syrian hamsters. J Pineal Res 2(4):325-330, 1985.

Vaughan GM, Shirani KZ, Ziegler MG, Pruitt BA Jr, and Mason AD Jr: The burned hamster as a model of nonthryoidal illness.

Proceedings of the 67th Annual Meeting of the Endocrine Society (Abstract 1281):321, 1985.

Vaughan GM, Taylor TJ, Lasko J, Bullard JJD, Coggins SH, Brown AC, and Mason AD Jr: The human melatonin rhythm and lack of influence by sympathetic activation. Adv Biosci 53:37-40, 1985.

Vaughan GM, Taylor TJ, Pruitt BA Jr, and Mason AD Jr: Pineal function in burns: melatonin is not a marker for general sympathetic activity. J Pineal Res 2(1):1-12, 1985.

Vaughan GM, Vaughan MK, and Mason AD Jr: Correlation of thyroid hormones and testes weight across pineal-related perturbations. Adv Biosci 53:257-264, 1985.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: An Analysis of the Adaptation of Renal Function Following Burn Injury

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

James C. McKay, MD, Major, MC
Calvin R. Kennedy
Arthur D. Mason, Jr., MD
George M. Vaughan, MD, Lieutenant Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S16277A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: An Analysis of the Adaptation of Renal Function Following Burn Injury

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: James C. McKay, MD, Major, MC
Calvin R. Kennedy
Arthur D. Mason, Jr., MD
George M. Vaughan, MD, Lieutenant Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Thermal injuries place great demands on the homeostatic mechanisms responsible for maintenance of fluid, electrolyte, and acid-base status and excretion of both metabolic waste products and drug metabolites. Kidney function is critical to all these processes. This study was designed to describe the response of the kidney to thermal injury and burn treatment procedures and define mechanisms of renal adaptations to trauma. Two patients have been enrolled in this study to date. The major emphasis has been an adequate assessment of glomerular filtration rate in these patients. A comparison of endogenous creatinine clearance to inulin clearance and that obtained by a single subcutaneous injection of I-iothalamate will be studied.

BETA-2-MICROGLOBULIN
BURNS
GLOMERULAR FILTRATION RATE
HUMAN
KIDNEY
POTASSIUM
PROTEINURIA
RENAL
SODIUM
THERMAL INJURY
VOLUNTEER

AN ANALYSIS OF THE ADAPTATION OF RENAL FUNCTION FOLLOWING BURN INJURY

INTRODUCTION

Burn injury and its therapy place great demands on the homeostatic mechanisms responsible for maintenance of fluid, electrolyte, and acid-base status and excretion of both metabolic and drug waste products. The function of the kidneys is critical to all these processes. Therefore, it is surprising that renal function in the postburn period has received scant attention (1-13).

---

[1] Loirat P, Rohan J, Baillet A, Beaufils F, David R, and Chapman A: Increased glomerular filtration rate in patients with major burns and its effect on the pharmokinetics of tobramycin. N Eng J Med 299:915-919, 1978.

[2] O'Neill JA Jr, Pruitt BA Jr, and Moncrief JA: Studies of renal function during the early postburn period. In: Research in Burns. Matter P, Barclay TL, and Konickova Z (eds). Bern: Hans Huber Publishers, c1971, pp 95-99.

[3] Haynes BW, DeBakey ME, and Denman FR: Renal function studies of severely burned patients: a preliminary report. Ann Surg 134:617-625, 1951.

[4] Sevitt S: Distal tubular necrosis with little or no oliguria. J Clin Path 9:12-30, 1956.

[5] Graber IG and Sevitt S: Renal function in burned patients and its relationship to morphological changes. J Clin Path 12:25-44, 1959.

[6] Eklund J, Granberg PO, and Liljedahl SO: Studies on renal function in burns. I. Renal osmolal regulation, glomerular filtration rate, and plasma solute composition related to age, burned surface area, and mortality probability. Acta Chir Scand 136:627-640, 1970.

[7] Eklund J: Studies on renal function in burns. II. Early signs of impaired renal function in lethal burns. Acta Chir Scand 136:735-740, 1970.

[8] Eklund J: Studies on renal function in burns. III. Hyperosmolal states in burned patients related to renal osmolal regulation. Acta Chir Scand 136:741-751, 1970.

[9] Vertel RM and Knochel JP: Nonoliguric acute renal failure. JAMA 200:598-602, 1967.

[10] Davies DM, Pusey CD, Rainford DJ, Brown JM, and Bennett JP: Acute renal failure in burns. Scand J Plast Reconstr Surg 13:189-192, 1979.

[11] Planas M, Wachtel T, Frank H, and Henderson LW: Characterization of acute renal failure in the burned patient. Arch Intern Med 142:2087-2091, 1982.

The studies which have been conducted had various limitations, i.e., studied only once at random times postburn (1), studied only during the first week postburn (2), small number of patients (3), prior renal function not defined (4-8), no comment on the development of nonrenal complications which can affect renal function (4-8), study limited to patients who developed renal failure (9-11), and summary article without original data (12-13). There have been no studies of proximal tubular renal function.

This study was proposed to define the normal physiologic adaptation of renal function to burn injury. The objectives of the study are to measure glomerular and tubular functions in patients suffering at least 30-percent total body surface area burns who had normal renal, hepatic, and cardiovascular function prior to burn injury. The patients will be followed sequentially from admission until death or discharge. Specific goals of the study include:

1. Determining glomerular filtration rate (GFR) and determining whether endogenous creatinine clearance is an accurate measure of GFR. In the literature, it is stated that GFR postburn is decreased, normal, or increased. Some of the differences probably relate to the time postburn when the measurements were made and some of the measurements may have been made in patients with complications (sepsis, hypotension, myocardial infarction, aminoglycoside antibiotic therapy, etc.) which depress renal function. From a literature review, it appears that fewer than 15 to 20 patients (1-3,5) have had creatinine clearance validated by simultaneous inulin clearance measurements and all but two (5) of these measurements were performed in patients with normal renal function. Only five to 10 patients (1) have had creatinine clearance verified by the glofil technique and all these patients had normal renal function. The importance of verifying the endogenous creatinine clearance by another technique is that the creatinine assay is nonspecific and interference can be produced by hyperalbuminemia, lipemia, hemolysis, bilirubinemia more than 50 milligrams per deciliter, elevated acetoacetate, ascorbic acid, or cephalosporin antibiotics (14). An accurate GFR is of critical importance clinically since dosages of many drugs, including aminoglycosides, are based upon the GFR.

---

[12]Sevitt S: Renal function after burning. J Clin Path 18:572-578, 1965.

[13]Gellman DD: The renal complications of burns. Canad Med Ass J 97:440-444, 1967.

[14]IL TEST Creatinine, Category Number 35164, Instrumentation Laboratories, Inc., 1980.

2. Defining the pattern of proteinuria and, specifically, the fractional excretion of beta-2-microglobulin. In the literature, there are few reports of proteinuria postburn (15-17). One states that all patients suffering a "large" burn will have transient proteinuria exceeding 0.55 grams per 24 hours from postburn days five through seven (15). Beta-2-microglobulin is freely filtered at the glomerulus and normally 98 percent is reabsorbed in the proximal tubules. To our knowledge, there has been no prior study of beta-2-microglobulin excretion in burned patients. By defining the excretion of beta-2-microglobulin, an index of proximal tubular function can be established. It will then be possible to study the problem of low sodium fractional excretion (FeNa) renal failure in burned patients. High Feb-2-microglobulin excretion would imply that the proximal tubule is damaged and the low FeNa is secondary to increased distal reabsorption. If the Feb-2-microglobulin is low, it would imply that the proximal tubule is functioning normally and that the lesion is either prerenal or glomerular.

3. Defining the excretion of phosphorous and calcium. To our knowledge, this has never been done, and recently, we have observed several patients with low serum concentrations of both calcium and phosphorous. Since calcium (ionized) and phosphorous are required for proper function of many organ systems, it is important to determine if there is excessive excretion or merely decreased intake so that proper replacement therapy can be given.

4. Defining the serum concentration of parathyroid hormone (PTH). PTH secretion is controlled by the serum concentration of ionized calcium and requires a permissive concentration of magnesium. PTH affects many organ systems, especially bone. If hypocalcemia is indeed a common occurrence postburn, then elevated PTH levels should result. Bedrest tends to cause excessive resorption and elevated PTH levels would accelerate the resorption.

5. Determining if the excessive renal potassium excretion is secondary to increased aldosterone secretion. This will require determination of sodium and potassium balance, renin, and aldosterone. Two studies have shown

---

[15]Shakespeare PG, Coombes EJ, Hambleton J, and Furness D: Proteinuria after burn injury. Ann Clin Biochem 18:353-360, 1981.

[16]Coombes EJ, Shakespeare PG, and Batstone GF: Urine proteins after burn injury. Clin Chim Acta 95:201-209, 1979.

[17]Eades CH Jr, Pollack RL, and Hardy JD: Thermal burns in man. IX. Urinary amino acid patterns. J Clin Invest 34:1756-1759, 1955.

elevated secretion of aldosterone in burned patients. If our balance studies show a correlation between the levels of aldosterone and potassium excretion, then further studies will be required to separate a primary from a secondary effect. The studies would use two drugs, one to block sodium entry into the tubules to determine the sodium dependence of the potassium excretion; the second drug would be an aldosterone inhibitor.

MATERIALS AND METHODS

A maximum of 25 patients age 18 or older admitted to this Institute who are admitted within 48 hours of injury, have at least a 30 percent burned body surface area, have no prior history of renal dysfunction, have no prior history of mycardial dysfunction (a history of mild treated hypertension is allowed), have a serum creatinine less than 1.4 milligrams per deciliter after initial resuscitation, and have no evidence of ascites will be entered in the study if they provide written informed consent. Patients are selected to include a spectrum of burn size and age.

While patients are in the intensive care unit at this Institute, all intake and output are recorded, with particular attention to sodium and potassium, calcium, and phosphorous. During each 24-hour period, all urine is collected and saved for chemical analyses. At the time of routine daily blood sampling, an additional maximum of 30 milliliters of blood is obtained for research analyses.

As soon as the ward physician determines that the patient has been stabilized, the GFR is determined by endogenous creatinine clearance and compared against the inulin and glofil (^{125}I-iothalamate, 20 millicuries) methods (18). The inulin GFR may be performed as often as once a week while the patient remains in the intensive care unit. The glofil GFR is be repeated if the patient's serum creatinine increases one milligram per deciliter. The patient's urine output must be greater than 30 milliliters per hour for the inulin or glofil GFR measurements to be performed.

After the patient is transferred to the intermediate care ward, a 24-hour urine and a maximum of 30 milliliters of blood is collected once per week. A final inulin and endogenous creatinine GFR is determined immediately prior to discharge.

Urine assays include sodium, potassium, chloride, urine urea nitrogen, creatinine, osmolality, phosphorous, calcium,

---

[18]Israelit AH, Long DL, White MG, et al: Measurement of glomerular filtration rat utilizing a single subcutaneous injection of ^{125}I-iothalamate. Kidney Int 4:346-349, 1973.

total protein, albumin, immunoglobulin G, and beta-2-microglobulin. In addition, selected samples will be analyzed by electrophoresis and isoelectrofocusing.

Serum assays include sodium, potassium, chloride, blood urea nitrogen, creatinine, osmolality, total protein, albumin, phosphorous, calcium (total and ionized), beta-2-microglobulin, renin, angiotensin II, aldosterone, parathyroid, and antidiuretic hormone. Selected samples will be analzyed by electrophoresis and isoelectrofocusing.

Clinical parameters monitored include weight, blood pressure, medications, percentage body surface area covered and type covering, and complications such as cardiac, pulmonary, sepsis, survival/death, etc.

It is expected that these patients will be grouped, at least, as uncomplicated/survived, complicated/survived, and complicated/died. If enough patients are available, complications may be analyzed separately, i.e., cardiac, pulmonary, sepsis, etc. The analysis of data will use multiple correlation-regression techniques.

RESULTS

To date, two patients have been enrolled in this study. The following observations were made:

1. Measurements of GFR by endogenous creatinine clearance and I-iodothalamate (glofil) were comparable.

2. The pattern of proteinuria, specifically the fractional excretion of beta-2-microglobulin suggests a proximal tubular defect with large quantities of beta-2-microglobulinuria. Such a proximal tubular defect does not, alone, explain the low urinary sodium and low fractional excretion of sodium seen in these patients in the postresuscitative period.

3. This preliminary study has also shown that despite low levels of plasma calcium, the ionized calcium fraction, which is the physiologically active moiety, was normal, suggesting that despite low total plasma calcium (even considering the presence of hypoalbuminemia), calcium supplementation is unnecessary. In addition, there were noted two isolated peaks in the parathyroid hormone level, both N-terminal and midmolecule, which bore no apparant correlation to plasma calcium or ionized calcium values.

4. The excessive renal potassium wasting seen in the postresuscitative period appears to be independent of the

renin-angiotensin-aldosterone axis, as these were in the normal range.

5. Measurements of catecholamines, metanephrines, and vanillylmandelic acid were consistently within the normal range throughout the study period.

6. Isolated elevation of antidiuretic hormone with hyponatremia suggested the syndrome of inappropriate antidiuretic hormone secretion or a reset osmostat (SIADH variant) as causal for the hyponatremia experienced by these patients.

DISCUSSION

As more patients are enrolled and studied, the significance of the above may become apparent. Further, it is expected that patients will be stratified into the respective groups and data analyzed by multiple correlation-regression techniques.

PRESENTATIONS/PUBLICATIONS

McKay JC: Renal adaptation following burn injury. Presented to the Renal Grand Rounds, Brooke Army Medical Center, February 1986.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: Anesthesiology

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 January 1985 - 31 December 1985

INVESTIGATORS

Steven I. Schmidt, MD, Major, MC
Charles P. Kingsley, MD, Captain, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: Anesthesiology

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Jan 85 through 31 Dec 85

INVESTIGATORS: Steven I. Schmidt, MD, Major, MC
Charles P. Kingsley, MD, Captain, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

During the period of this report, 388 anesthetics were administered to 133 patients, an average of 2.92 anesthetics per patient. The most commonly used anesthetic agent was enflurane (71.91 percent), followed by ketamine (13.40 percent) and isoflurane (9.02 percent). Due to the nature and combinations of procedures now performed, regional anesthesia is no longer used.

ANESTHESIOLOGY

PREOPERATIVE PROCEDURES

Evaluation. Most burn patients are several days postinjury when first seen by the anesthesiologist. In the immediate postburn period, time is used to gain abundant physiologic data from routine monitoring of various indices, i.e., hematologic (hematocrit, electrolytes, liver and renal function tests), pulmonary (arterial blood gases, respiratory rate, chest roentgenograms), cardiovascular (blood pressure, central venous pressure, cardiac output), and renal (urine output, urine chemistry), in addition to the usual preoperative patient interview and physical examination. All patients, regardless of age, who have electrical injuries are required to have a preoperative electrocardiogram performed to rule out possible myocardial damage.

Preparation. All patients are placed on "nothing by mouth" status after 2400 hours the day prior to surgery with the exception of children, who may receive clear liquids up to five hours prior to surgery. Due to extraordinary fluid requirements in most burn patients, an intravenous infusion, if not already in place, is begun the evening prior to surgery.

Premedication. Glycopyrrolate (Robinul^{R}), 0.005 milligrams per kilogram body weight to a maximum dose of 0.4 milligrams, is given intramuscularly 30 minutes prior to anesthesia or intravenously upon entering the operating room. No other premedications are routinely used with the exception of diazepam preceding ketamine anesthetic.

Fluids. All fluids, except hyperalimentation solutions, are changed to five-percent glucose in water with Ringer's lactate or Ringer's lactate on arrival in the operating room. Hyperalimentation solutions are continued throughout operative procedures.

TYPES OF ANESTHESIA

The pattern of anesthetic administration has changed from previous years and involves a greater use of enflurane, which has been used in more than 60 percent of all anesthetic procedures. Ketamine, halothane, and isoflurane are used, but to a much lesser extent (Table 1).

Enflurane (Ethrane^{R}). Enflurane is a halogenated ether which provides rapid induction and good muscle relaxation. Biotransformation amounts to two to 2.5 percent of an inhaled dose, which perhaps accounts for the few clinical toxic effects observed. Plasma fluoride levels in hypermetabolic burn

TABLE 1

PATTERN OF ANESTHESIA ADMINISTRATION

Agent	1982		1983		1984		1985	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Enflurane	335	62.97	184	63.23	290	62.91	279	71.91
Halothane	1	0.19	0	0.00	0	0.00	2	0.52
Isoflurane	0	0.00	0	0.00	0	0.00	35	9.02
Nitrous Oxide	8	1.50	22	7.56	27	5.86	0	0.00
Ketamine	169	31.77	66	22.68	88	19.09	52	13.40
Local	15	2.81	13	4.47	14	3.04	10	2.58
Other	4	0.75	6	2.00	42	9.11	10	2.58
TOTAL	532	100.00	291	100.00	461	100.00	388	100.00

patients during and after enflurane administration have been measured and found not to be in the toxic range. Enflurane is presently the most commonly used anesthetic agent at this Institute.

Halothane (Fluothane^{R}). Halothane is a halogenated alkane that has met with only limited use over the last four years. Biotransformation can account for as much as 25 percent of an inhaled dose. Halothane hepatitis, although rare, fortunately has not been reported in burn patients. Since the successful introduction of enflurane, few indications for halothane's use exist in this patient population, which may be predisposed to hepatitis from multiple transfusions with blood products. However, its use is indicated primarily in the burned pediatric patient who requires an airway be secured by an endotracheal tube. Halothane hepatitis has not been reported to be an issue in the pediatric population.

Isoflurane (ForaneR). Isoflurane, which is an isomer of enflurane, is the most recent halogenated ether to be introduced. Biotransformation amounts to only 0.25 percent of an inhaled dose and no toxic reactions to the metabolic products have been reported to date. It has a rather pungent odor that tends to limit its use for inhalational induction. It is noted for producing minimal myocardial depression and a marked reduction in systemic vascular resistance. At this time, isoflurane has found limited use at this Institute, but as more experience with this agent is gained, its use will probably increase.

Nitrous Oxide. This agent is used in concentrations of 50 to 60 percent with oxygen. It is used mainly in conjunction with other analgesic or anesthetic agents.

Ketamine. This agent is used both intramuscularly and intravenously to produce its characteristic dissociative state with preservation of basal functions and laryngeal reflexes plus stimulation of the cardiovascular system. Unfortunately, ketamine shares with its parent compound, phenycyclidine, the production of a high incidence of unpleasant hallucinogenic side effects. There seems to have been a "batch" difference in ketamine and that possessed by this Institute in the past has produced an almost 100-percent incidence of these side effects. New methods of administering the drug as well as various methods of premedication and patient preparation appear to have reduced the unpleasant emergence reactions to a level where they are of little consideration in the well-selected patient. Laryngospasm, airway obstruction, and regurgitation can occur with ketamine. Pronounced blepharospasm prevents its use in eye cases. All ketamine anesthetics, other than in children, are preceded by intravenous administration of diazepam (0.15 to 0.2 milligrams per kilogram body weight).

Succinylcholine. Succinylcholine has not been used for any purpose at this Institute for more than nine years.

Regional Anesthetics. Regional anesthesia is generally considered one of the safest methods available, but its use in the thermally injured patient is limited for several reasons. Sepsis and infection of the skin over or near the site of injection are contraindications for use and multiple-site operations also limit the practicality of this method.

MONITORING TECHNIQUES

Cardiovascular System. Monitoring includes the precordial and/or esophageal stethoscope, peripheral pulse, blood pressure, central venous pressure, Swan-Ganz catheter, electrocardiogram, and urine output.

The Dinamap™ blood pressure instrument is routinely used for intraoperative blood pressure monitoring. Since it can be used over dressings and is noninvasive, it is a most practical method of monitoring blood pressure in our patient population. Usually, blood pressure is monitored at two sites. Direct arterial lines are used when necessary.

Respiratory System. Monitoring includes rate, auscultation, arterial blood gases, pulmonary functions (pre and intraoperative), hemoglobin oxygen saturation, and end tidal carbon dioxide. During the past year, the introduction of new noninvasive monitors has made a significant contribution to the management of the thermally injured patient. The measurement of hemoglobin oxygen saturation by pulse oximetry, end-tidal carbon dioxide, and pulmonary function parameters all represent no risk to the patient, are easily obtainable, and are accurate. These monitors have become standard in our anesthetic care of the burned patient.

Body Temperature. In most cases, a temperature monitor is employed. Because of the greatly increased evaporative losses in burn patients, hypothermia is a serious problem. Several methods are employed to maintain body temperature during anesthesia. Ambient temperatures were maintained between 82 and 87° F in the past; however, patient cooling still occurred. Maintaining the room temperature above 88° F appears to have corrected the problem. Anesthetic gases are heated and humidified. Radiant heat lamps used alone have been found to have little effect in preventing patient cooling. The K-thermia heating blanket is also sometimes used. It is probably used most effectively on children weighing less than 10 kilograms and on febrile patients.

RESULTS

Complications. There was one cardiac arrest due to an intraoperaive myocardial infarction. The patient was a 59-year old white female with a 49-percent total body surface area burn, of which 47 percent was full-thickness. She was a chronic schizophrenic who was comatose and ventiiator-dependent. At approximately 30 days postburn while undergoing tangential excision of her back in the prone position, the myocardial event occurred. The patient expired two hours postoperatively.

Patient Data. Tables 2 and 3 provide overall anesthetic patient data.

Operative Procedures. Table 4 illustrates recent trends in operative procedures.

TABLE 2

FREQUENCIES OF USE FOR SELECTED INTRAOPERATIVE MONITORS/PARAMETERS

Monitor/Parameter	Number of Intraoperative Uses	Percent of Total Anesthetics
Dinamap^{R} (Blood Pressure)	388	100.00
Electrocardiogram	388	100.00
End-tidal Carbon Dioxide	313	80.67
Inspired Oxygen Concentration	310	79.90
Pulmonary Function	277	71.39
Pulse Oximeter (Hemoglobin Saturation)	251	64.69
Central Venous Pressure	26	6.70
Arterial Line	25	6.70
Swan-Ganz Catheter	13	3.35

TABLE 3

OVERALL ANESTHETIC PATIENT DATA - 1971 THROUGH 1985

Year	Number of Patients	Number of Patients Anesthetized	Percentage of All Patients	Total Anesthestics Given	Average Anesthetics Per Patients Anesthetized
1971	301	179	59.47	475	2.65
1972	301	183	60.80	575	3.14
1973	273	141	51.65	377	2.67
1974	226	123	54.42	380	3.09
1975	254	142	55.91	490	3.45
1976	277	139	50.18	476	3.42
1977	242	129	53.30	344	2.67
1978	268	151	56.34	435	2.88
1979	267	161	60.30	554	3.44
1980	243	148	60.91	531	3.59
1981	208	127	61.06	404	3.18
1982	231	151	65.37	532	3.52
1983	179	98	54.75	291	2.97
1984	190	139	73.16	461	3.32
1985	197	133	67.51	388	2.92

TABLE 4

RECENT TRENDS IN OPERATIVE PROCEDURES

Procedure	1981		1982		1983		1984		1985	
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Excision	212	36.68	257	33.29	196	42.06	323	41.04	304	43.37
Autograft	293	50.69	405	52.46	203	43.56	371	47.14	304	43.37
Orthopedic	23	3.98	31	4.01	22	4.72	30	3.81	19	2.71
Chrondrectomy	3	0.52	0	0.00	2	0.43	4	0.51	0	0.00
Eye and Lid	3	0.52	14	1.81	8	1.72	18	2.29	9	1.28
Intra-abdominal	1	0.17	6	0.78	2	0.43	5	0.64	12	1.71
Plastic	3	0.52	15	1.94	2	0.43	5	0.64	9	1.28
Other	40	6.92	44	5.70	31	6.65	31	3.94	44	6.28
TOTAL	578	100.00	772	100.00	466	100.00	787	100.00	701	100.00

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: Antithrombin III Deficiency in Thermally Injured Patients

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Stephen M. Pratt, MD
Robert C. Allen, MD, PhD, Major, MC
Anthony A. Smith, MD, Major MC
Michael Berkland, MD, Captain, MC*
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

*Department of Pathology
Brooke Army Medical Center
Fort Sam Houston
San Antonio, Texas 78234-6200

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: Antithrombin III Deficiency in Thermally Injured Patients

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 85

INVESTIGATORS: Stephen M. Pratt, MD
Robert C. Allen, MD, PhD, Major, MC
Anthony A. Smith, MD, Major, MC
Michael D. Berkland, MD, Captain, MC
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Antithrombin III has been documented as the most important naturally occurring inhibitor of thrombosis and low levels have been shown to predispose to thrombotic events (1-3). However, few studies of antithrombin III and other plasma factors effecting hemostasis in burn patients have been reported. Analysis of six of our most recent patients with angiographically documented pulmonary emboli identified low antithrombin III levels by functional and/or immunologic assay in four patients. In this group, emboli were documented 35 to 100 days postburn (mean = 44 days); average total body surface area burned was 57 percent. Two of these patients sustained recurrent pulmonary emboli while still on heparin therapy and all patients required greater than 1,800 units of heparin per hour to maintain therapeutic anticoagulation as judged by partial thromboplastin time of 1.5 to 2.5 times normal, an amount significantly higher than the clinical norm.

A prospective review is thus being undertaken to identify coagulation factor abnormalities in burn patients. Fifty patients admitted to the Institute will have antithrombin III, protein C, and protein S levels determined. Data will be correlated with clinical and laboratory evidence of hypercoagulability.

ANTITHROMBIN III DEFICIENCY IN THERMALLY INJURED PATIENTS

INTRODUCTION

A wide array of conditions not infrequently encountered in clinical medicine have been classified as hypercoagulability disorders. These include deep venous thrombosis, pulmonary emboli, abnormal tendency for graft thrombosis, and generally any clinical state in which there exists enhanced coagulation and inappropriate thrombosis (4). However, hypercoagulability remains a rather imprecisely defined concept. While much is known of specific factors, which when deficient lead to inappropriate bleeding diathesis, there exists a deficiency in our knowledge of factors which serve to regulate ongoing intravascular coagulation. Two systems now appear to be important in this back regulation. Both are regulated by proteins which can now be measured by functional and antigenic assays.

The Antithrombin III System. Antithrombin III (AT3) is an alpha-2 globulin which has a molecular weight of 65,000 and a half-life of 2.8 days (5). A serine protease inhibitor, AT3 has a direct inhibitory effect on thrombin. Also inhibited are factors IXa, Xa, XIa, and XIIa (2). Low levels may be congenital (6) or acquired (7) and have been shown to

---

[1]Kimball DB and Schialla S: Normal hemostasis and hemorrhagic disorders in vascular occlusive disorders. Collins FJ Jr (ed). In Medical and Surgical Management. New York: Futura Publishing Company, 1981, p 8.

[2]Mammen EF, Miyakawa T, Phillips TF, et al: Human antithrombin concentrates and experimental disseminated intravascular coagulation. Semin Thromb Hemost 11:373-383, 1985.

[3]Rao AK, Niewarowski S, Guzzo J, et al: Antithrombin III levels during heparin therapy. Thrombosis Research 24:181-186, 1981.

[4]Collins GJ Jr., Scialla S, and Kimball DB Jr: Enhanced coagulation and inappropriate thrombosis: hypercoagulability. Collins FJ (ed). In Medical and Surgical Management. New York: Futura Publishing Company, 1981, pp 27-67.

[5]Abildgaard U, Fagerhol MK, and Egeberg O: Comparison of progressive antithrombin activity and the concentration of three thrombin inhibitors in human plasma. Scand J Clin Lab Invest 26:349-354, 1970.

[6]Von Kaulla E and Von Kaulla KN: Deficiency of antithrombin 3 activity associated with hereditary thrombosis tendency. J Med (Basel) 3:349-58, 1972.

[7]Bick RL: Clinical relevance of antithrombin III. Semin Thromb Hemost 8:276-287, 1982.

predispose to episodes of inappropriate intravascular coagulation.

The activity of AT3 on thrombin is accelerated by heparin (8), and thus it has also been termed the "heparin cofactor." The heparin-AT3 complex also inactivates factor VIIa (9). Congenital AT3 deficiency has been documented in patients with recurrent deep venous thrombosis (10-11), pulmonary emboli (12), dialysis fistula failure (13), and mesenteric venous occlusion (14-15). AT3 has also been demonstrated as a cause of arterial graft thrombosis (16-17). Acquired defects have been linked to protein deficiency states (6), such as

---

[8] Rosenberg RD and Damus PS: The purification and mechanism of action of human antithrombin-heparin cofactor. J Biol Chem 248:5490-6505, 1973.

[9] Godal HC, Rygh M, and Laake K: Progressive inactivation of purified factor VII by heparin and antithrombin III. Thromb Res 5:773-775, 1974.

[10] Johansson L, Hedner U, and Nilsson IM: Familial antithrombin III deficiency as pathogenesis of deep venous thrombosis. Acta Med Scand 204:491-495, 1978.

[11] Machie M, Bennett B, Ogston D, et al: Familial thrombosis: inherited deficiency of antithrombin III. Br Med J 1:136-138, 1978.

[12] Rothschild BM. The role of antithrombin III in clinical management of pulmonary embolization. Am J Med 74: 529-531, 1983.

[13] Kauffman HM Jr, Ekbom GA, Adams MB, et al: Hypercoagulability: a cause of vascular access failure. Proc Clin Dial Transplant Forum 9:28-31, 1979.

[14] Gruenberg JC, Smallridge RC, and Rosenberg RD: Inherited antithrombin-III deficiency causing mesenteric venous infarction: a new clinical entity. Ann Surg 181:791-794, 1975.

[15] Karl R, Garlick I, Zarins C, et al: Surgical implications of antithrombin III deficiency. Surgery 89:429-433, 1981.

[16] Towne JB, Bernhard VM, Hussey C, et al: Antithrombin III deficiency - a cause of unexplained thrombosis in vascular surgery. Surgery 89:735-742, 1981.

[17] McDaniel MD, Pearce WH, Yao JST, et al: Sequential changes in coagulation and platelet function following femorotibial bypass. J Vasc Surg 1:261-268, 1984.

inappropriate catabolism, poor nutritional states (18), the nephrotic syndrome (19), and liver failure (20), consumptive conditions including disseminated intravascular coagulation (21-22), sepsis (23), and shock (24), and in trauma (25) and burn patients. Heparin in one recent report has been shown to decrease circlating levels of AT3, a finding which could have significant implications in the period of conversion from intravenous heparin therapy to oral agent anticoagulation (3). Prognostic implications of AT3 in septic patients have also been cited with levels which fall and remain low, portending a worse outcome (26-27).

The Protein C System. Protein C is a two-chain glycoprotein (molecular weight = 62,000) (28) which exerts its effect on the coagulation cascade by inhibiting factors VIIIa

---

[18]Flinn WR, McDaniel MD, Yao JST, et al: Antithrombin III deficiency as a reflection of dynamic protein metabolism in patients undergoing vascular reconstruction. J Vasc Surg 1:888-894, 1984.

[19]Kauffmann RH, Veltkamp JJ, Van Tilburg NH, et al: Acquired antithrombin III deficiency and thrombosis in the nephrotic syndrome. Am J Med 65:607-613, 1978.

[20]Henson A, Loeliger E: Antithrombin III: its metabolism and function. Thromb Diath Haemorr (Suppl) 9:1-84, 1973.

[21]Bick RL, Dukes ML, Wilson WL, et al: Antithrombin III (AT-III) as a diagnostic aid in disseminated intravascular coagulation. Thromb Res 10: 721-729, 1977.

[22]Bick RL, Bick MD, and Feketo LF: Antithrombin III patterns in disseminated intravascular coagulation. Am J Clin Pathol 73: 577-583, 1980.

[23]Schipper HG, Roos J, v.d. Meulen F, et al: Antithrombin III deficiency in surgical intensive care patients. Thromb Res 21:73-80, 1981.

[24]Blauhut B, Necek A, Kramar H, et al: Activity of antithrombin III and effect of heparin on coagulation in shock. Thromb Res 19:775-782, 1980.

[25]Boyter AE, Seaber AV, Dombrose FA, Urbaniac JR, et al: Coagulation changes in elective surgery and trauma. Ann Surg 193:210-213, 1981.

[26]Wilson RF, Mammen EF, Robson MC, et al: Antithrombin, prekallikrein, and fibronectin levels in surgical patients. Arch Surg 121:639-640, 1986.

[27]Lammle B, Tran TH, Ritz R, et al: Plasma prekallikrein, factor XII, antithrombin III, C_1-inhibitor, and α_2-macroglobulin in critically ill patients with suspected disseminated intravascular coagulation (DIC). Am J Clin Pathol 82:398-404, 1984.

[28]Kisiel W: Human plasma protein C: isolation, characterization, and mechanism of activation by alpha-thrombin. J Clin Invest 64:761-769, 1979.

and Va (29). Thrombin production, and ultimately fibrin formation, is thus limited by the inhibition of actived factor X. A second mechanism of activity attributed to the protein C System has recently been ellucidated. Protein C has been shown to increase circulating levels of plasminogen activator, a factor necessary for clot lysis (30). Thus, protein C has both anticoagulation and fibrinolytic activity.

Protein C must initially be converted to an active form (protein Ca), an in vivo process which has recently been described. Thrombin initiates this process by binding to an endothelial cell protein, thrombomodulin. This thrombin-thrombomodulin complex, in the presence of calcium, rapidly effects the activation of protein C (31). The subsequent inhibition of factor Va by protein Ca is greatly enhanced by yet another factor, protein S, which is also found in serum in bound and free form (32).

Acquired and congenital deficiencies in protein C and protein S are now being reported and the importance of abnormalities in this system to hypercoagulability states is just being realized.

Coagulation Abnormalities in the Burn Patient. While hemorrhagic disorders in burn patients have formed the subject of numerous reports (33), hypercoagulation from a hematologic aspect has been given relatively little attention. AT3 levels in burn patients are reported in a 12-patient study (34), but levels of protein C and protein S have not been addressed in burn patients. However, the sequelae of hypercoagulation, deep

---

[29] Marlar RA, Kleiss AJ, and Griffin JH: Mechanism of action of human activated protein C, a thrombin-dependent anticoagulant enzyme. Blood 59:1067-1072, 1982.

[30] Van Hinsberg VWM, Bertina RM, Van Wijngaarden A, et al: Activated protein C decreases plasminogen activator-inhibitor activity in endothelial cell-conditioned medium. Blood 65:444-451, 1985.

[31] Clouse LH and Comp PC: The regulation of hemostasis: the protein C system. N Engl J Med 314:1298-1304, 1986.

[32] Egeberg O. Inherited antithrombin deficiency causing thrombophilia. romb Diath Haemorrh 13:516-530, 1965.

[33] Ono I, Ohura T, Hamamoto J, et al: Clinical observations of coadministration of antithrombin III preparation with heparin in burned patients. JBCR 5:25-29, 1984.

[34] Gehrke CF, Penner JA, Niederhuber J, and Feller I: Coagulation defects in burned patients. Surg Gyn Obstet 133:613-616, 1971.

venous thrombosis (35-36), and pulmonary emboli (37) have been addressed by several authors with varying reports as to prevalence. Autopsy diagnosis of pulmonary emboli has been documented in one study to be 30.2 percent (38), a result that agrees with a 29-percent incidence seen clinically by others (14). Still, further reports note a significantly lower incidence. Similarily, the incidence of deep venous thrombosis varies between reports (39).

Specific factors which might predispose to these potential causes of morbidity and mortality have yet to be defined. The goal of the present study is to define "normal" levels of AT3, protein C, and protein S in burn patients and to identify any increased risk of thromboembolic sequaelae associated with abnormal circulating levels of these particular factors.

MATERIALS AND METHODS

After obtaining informed consent, 50 consecutive adult volunteers who have been admitted to the Institute will receive admission, weekly, and clinically indicated determinations of AT3, protein C, protein S, complete blood count, disseminated intravascular coagulation screen, urine protein, and SMAC 18. Additionally, a record will be maintained of the patient's clinical course and weekly assessments of peripheral vascular status will be assessed by noninvasive means. Particular attention will be given to episodes of sepsis, deep venous thrombosis, pulmonary emboli, operative procedures, and transfusion therapy. Nutritional support will also be noted.

RESULTS

Thirty-one patients have been entered into the study. Serum samples are being frozen and appropriate reagents and equipment to perform the necessary determinations have been ordered. These initial samples, after evaluation, will guide the direction of the remainder of the study.

Mayou BJ, Wee J, and Girling M: Deep vein thrombosis in burns. Burns 7:438-440, 1980.

Freeark RJ, Boswick J, and Fardin R: Posttraumatic venous thrombosis. Arch Surg 95:567-575, 1967.

Coleman JB and Chang FC: Pulmonary embolism: an unrecognized event in severely burned patients. Am J Surg 130:697-699, 1975.

Warden GD, Wilmore DW, and Pruitt BA Jr: Central venous thrombosis: a hazard of medical progress. J Trauma 13:620-626, 1973.

McDowall RA: Pulmonary embolism and deep vein thrombosis in burned patients. Br J Plast Surg 26:176-177, 1973.

PRESENTATIONS/PUBLICATIONS

None.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: Chemical Burns

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

David W. Mozingo, MD, Captain, MC*
Anthony A. Smith, MD, Major, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

*Dr. Mozingo is currently a resident in general surgery at the Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas 78234-6200

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: Chemical Burns

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: David W. Mozingo, MD, Captain, MC
Anthony A. Smith, MD, Major, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

In chemical skin injuries, reduction of the time of exposure to the causative agent and prompt recognition of systemic toxicity are necessary to lessen the severity of the insult, reduce morbidity, and maximize survival.

During a 17-year period (1969 to 1985), 87 (4.9 percent) of the 4,212 burn patients admitted to the US Army Institute of Surgical Research sustained chemical burns. Twelve patients died (13.8 percent). White phosphorous, the most common causative agent, produced cutaneous injury in 49 patients. Acids (13 patients), alkalies (10 patients), and organic solvents (five patients) were the other common causes of injury.

Initial treatment consisted of water lavage. Later wound management was carried out with topical antibiotic therapy and excision and grafting as necessary. Systemic toxicity due to phenol, nitrate, and formate absorption occurred, as did acute tubular necrosis following copper sulfate treatment of white phosphorous burns. Inhalation injury occurred in five patients. A decrease in hospital stay for chemically injured patients was observed.

To minimize tissue injury due to chemicals, clothing should be removed promptly and water lavage begun. Systemic toxicity and inhalation injury are rare, but often severe and increase mortality.

CHEMICAL BURNS

INTRODUCTION

Thousands of chemicals capable of causing skin and inhalation injuries are used in industry, agriculture, the military, science, and the home (1). Cutaneous injury from caustic chemicals is unlike cutaneous thermal injury in that ongoing tissue destruction occurs long after the initial exposure. Only prompt skin irrigation will lessen the depth of injury. Additionally, with significant absorption of the chemical, systemic toxicity can occur. This series represents not only one of the largest reports of chemical injuries, but also one of the few with a significant number of white phosphorous (WP) burns, which are rarely encountered in nonmilitary institutions.

MATERIALS AND METHODS

The clinical records of 4,212 consecutive patients admitted to the US Army Institute of Surgical Research from 1969 to 1985 were reviewed. Of these, 87 (4.9 percent) sustained chemical skin injury. There were 85 males and two females with an age range of nine to 59 years and a mean age of 27.8 years. Fifty-eight patients were active duty military and 29 were civilians.

The most common causative agent was WP, which injured 49 patients. Most of these injuries occurred in Vietnam and the clinical records did not always address the presence of WP in the wounds. Acids (13 patients), alkalies (10 patients), and organic solvents (five patients) were the other common chemicals causing injury (Table 1). A thermal component of skin injury, other than the heat produced by the chemical reaction at the skin surface, was identified in 13 of the 38 patients who were not burned by WP. Seven scald, four flame, and two freezing injuries occurred. The percent total body surface burned ranged from one to 90.5 with a mean burn size of 25.4 percent. Full-thickness injury ranged from zero to 90.5 percent, with a mean of 13.8 percent. Burn size was similar when patients sustaining thermal, WP, and other chemical burns were compared (Table 2). Systemic toxicity from cutaneous absorption occurred in two patients with phenol toxicity, one with formate toxicity, one with nitrate toxicity, and two from copper sulfate which was used to "neutralize" WP burns.

---

[1]Curreri PW, Asch MJ, and Pruitt BA Jr: The treatment of chemical burns: specialized diagnostic, therapeutic, and prognostic considerations. J Trauma 10:634-642, 1970.

TABLE 1

CHEMICAL AGENTS CAUSING INJURY

Chemical Agent	Number of Patients
White Phosphorous	49
Acids	13
Sulfuric (10) Formic (2) Hydrochloric (1)	
Alkalies	10
Sodium Hydroxide (3) Lye (2) Ammonia (1) Other (4)	
Organic Compounds	5
Phenols (2) Gasoline (1) Paint Thinner (1) Triethylene Glycol (1)	
Freon	2
Sodium Nitrate	2
Other	5
TOTAL	87

Thirty-five associated injuries occurred in 18 patients, with fractures being the most common (Table 3). Patients injured by WP were more likely to have associated injuries than those burned by other chemicals. Sixty-one patients required split-thickness cutaneous autografts (Table 4). Fifty-three patients required 112 other nongrafting surgical procedures including 20 amputations and 15 procedures involving the eye or periorbital tissue (Table 5). One enucleation was performed.

One hundred five complications occurred. Half were due to infection (Table 6). Cellulitis (nine), septicemia (eight), and pneumonia (seven) were the most common early complications. The most frequent late complication was contracture of one or

TABLE 2

BURN SIZE AND MORTALITY

Patient Group	Number of Patients	Total Burn Size	Third Degree Burn Size	Mortality
All Chemical Burns	87	25.4%	13.8%	13.8%
WP Burns	49	22.0%	11.2%	4.1%
Non-WP Chemical Burns	38	29.8%	17.8%	26.3%
All Burn Admissions	4,015	34.8%	15.6%	28.5%

TABLE 3

ASSOCIATED INJURIES

Associated Injury	WP Burns	Other Chemical Burns
Fracture	6	4
Traumatic Amputation of Limb	5	-
Laceration	4	1
Eye Injury	2	3
Inhalation Injury	2	3
Perforated Tympanic Membrane	1	1
Traumatic Amputation of Digits	1	-
Foreign Body in Knee Joint	1	-
Foreign Body in Sinus	1	-
TOTAL	23	12

TABLE 4

GRAFTING OPERATIONS

Grafting Procedure	Number of Cases
Homograft	268
Autograft	145
Porcine Xenograft	131

TABLE 5

MISCELLANEOUS OPERATIONS

Operation	WP Burns	Other Chemical Burns	Total
Debridement	27	16	43
Escharotomy/Fasciotomy	8	6	14
Amputation of Digits	12	-	12
Tarsorrhaphy	5	4	9
Tracheostomy	8	-	8
Chondrectomy	7	-	7
Open Reduction of Fracture	4	1	5
Arthrodesis	4	-	4
Contracture Release	4	-	4
Eyelid Release	4	-	4
Conjunctival Flap	1	-	1
Enucleation	-	1	1
TOTAL	84	28	112

TABLE 6

COMPLICATIONS

Complication	WP Burns	Other Chemical Burns	Total
Contracture of Joints	8	3	11
Cellulitis	3	6	9
Septicemia	5	3	8
Upper Gastrointestinal Bleeding	6	1	7
Pneumonia	5	2	7
Chondritis	6	-	6
Osteomyelitis	5	-	5
Burn Wound Infection	3	2	5
Ectropion	4	-	4
Acute Renal Failure	2	2	4
Blindness	1	2	3
Copper Sulfate Toxicity	2	-	2
Suppurative Thrombophlebitis	2	-	2
Superior Mesenteric Artery Syndrome	2	-	2
Myocardial Infarction	-	2	2
Phenol Toxicity	-	2	2
Pulmonary Embolus	-	2	2
Heterotopic Calcification	1	-	1
Brain Death	-	1	1
Formate Toxicity	-	1	1
Nitrate Toxicity	-	1	1
Pancreatitis	-	1	1
Other	12	7	19
TOTAL	67	38	105

more joints, which occurred in eleven patients. Patients with chemical burns, other than WP, had shorter hospital stays than the average burn patient admitted during the period of the study (Table 7). Twelve patients died (13.8 percent).

DISCUSSION

Patients with chemical skin injuries compose a small percentage of the patients treated in burn centers. These patients require prompt initial treatment, and the potential for systemic toxicity due to cutaneous absorption must be recognized. Since therapy is dependent upon recognition of the caustic agent, treatment begins with a good history. Curreri et al (1), Leonard et al (2), and others (3-7) have stressed the importance of early intervention to lessen tissue destruction and minimize the risk of systemic toxicity.

Initial Treatment. Prompt removal of clothing and copious irrigation with water should occur as soon as possible after contact with any caustic agent. Shoes must be removed since they may trap the agent against the skin, causing prolonged contact. The severity of the burn and the duration of hospital stay have both been shown to decrease when water lavage is initiated in the field (2). Experimental studies have also confirmed that less tissue destruction occurs with prompt and prolonged lavage. In animals burned by either acid or alkali, Gruber et al (8) showed an earlier return to preburn tissue pH if wounds were irrigated with water immediately postburn. In the case of sodium hydroxide burns, this normalization of pH may require continuous irrigation for up to one hour postburn. Lavage effectively dilutes the chemical in contact with the skin and washes off unreacted reagents not yet at the skin-chemical interface. No role exists for neutralizing acid and alkali injuries as time is wasted searching for specific

---

[2]Leonard LG, Scheulen JJ, and Munster AM: Chemical burns: effect of prompt first aid. J Trauma 22:420-423, 1982.

[3]Fitzpatrick KT and Moylan JA: Emergency care of chemical burns. Postgrad Med J 78:189-194, 1985.

[4]Jelenko C 3d: Chemicals that "burn." J Trauma 14:65-72, 1974.

[5]Pruitt BA Jr: The burn patient: I. Initial care. Curr Probl Surg 16:1-62, 1979.

[6]Rodeheaver GT and Edlich RT: Early management of chemical skin burns. Curr Concepts Trauma Care Spring:3-6, 1979.

[7]Walters MJ and Lowell GG: Corneal problems in burned patients. JBCR 3:367-370, 1982.

[8]Gruber RP, Laub DR, and Vistnes LM: The effect of hydrotherapy on the clinical course and pH of experimental cutaneous chemical burns. Plast Reconstr Surg 55:200-204, 1975.

TABLE 7

HOSPITALIZATION

	Patient Group	Average Hospital Days
1950 - 1968 (Curreri et al)*	All Burn Admissions	74
	Chemical Burns	104
1969 - 1985 (Mozingo et al)	All Burn Admissions	42
	Chemical Burns (WP + Non-WP)	67
	WP Chemical Burns	94
	Non-WP Chemical Burns	31

*See reference 1.

reagents and the exothermic reaction produced by neutralization may extend the depth of burn by increasing the temperature at the chemical-skin interface. Appropriate fluid resuscitation must begin promptly and tetanus prophylaxis must be administered, as in other thermally injured patients.

Associated Injuries. Fractures were the most common associated injury, occurring in ten patients. As in thermal injury, open fractures, or those requiring open reduction and located near the injured skin are at increased risk for infection (5). To minimize this risk, open reduction or occlusive casting should be avoided near chemically injured skin.

Thirteen patients, with cutaneous injury from agents other than WP, had a nonchemical thermal component of skin injury. Seven chemical solutions were heated and produced scald burns. Five flame burns complicated chemical injury when explosion or ignition of the chemicals occurred. Two patients contacted liquid freon, producing a cold exposure injury. When thermal injury is associated with chemical burns, there is increased depth and extent of skin destruction.

Of the five instances of documented inhalation injury, three were due to inhalation of aerosolized chemicals and two were due to smoke inhalation. Three of these patients died. In thermally injured patients, inhalation injury has been shown to increase mortality by up to 20 percent depending on the age

of the patient and extent of burn (9). When inhaled chemicals are absorbed, they may produce systemic toxicity (4,10).

Twenty-one percent of the patients in our series had injuries other than those related to direct contact with chemicals. The fact that these associated injuries frequently pose a greater threat to life or limb than the patient's burn underscores the importance of their diagnosis and appropriate treatment. Additionally, care of such injuries frequently requires hospitalization long after the chemical burn has healed.

Later Care of Chemical Burns. Following adequate lavage and debridement of chemically injured skin, the wounds were treated with topical chemotherapeutic agents. Prophylactic systemic antibiotics were not used. Prior to 1973, the eschar of full-thickness burns was usually allowed to separate, aided by daily debridement. The subeschar granulation tissue was then autografted with split-thickness skin. Since 1973, tangential and sequential burn wound excision as well as scapel excision at the level of the investing fascia (5,11-12) with subsequent split-thickness cutaneous autografting has been commonly employed.

Ocular Injuries. There were five globe injuries, three due to penetrating trauma and two from direct contact of the chemical with the eye. Two ocular chemical burns in our series resulted in blindness and one enucleation was performed for corneal perforation complicated by enopthalmitis. Ectropion was corrected with eyelid release in four patients. One patient with extensive chemical burn about the eye had total destruction of the lid margins and full-thickness injury to the remainder of the eyelid skin and a masquerade procedure (13) was required. Nine tarsorrhaphies were also performed to provide globe coverage to prevent dessication of the cornea and infection of the anterior chamber of the eye.

---

[9]Shirani KZ, Pruitt BA Jr, and Mason AD Jr: The influence of inhalation injury and pneumonia on burn mortality. Ann Surg 205:82-87, 1987.

[10]Simpson LA and Cruse CW: Gasoline immersion injury. Plast Reconstr Surg 67:54-57, 1981.

[11]Burke JF, Quinby WC Jr, and Bondoc CC: Primary excision and prompt grafting as routine therapy for the treatment of thermal burns in children. Surg Clin North Am 56:477-494, 1976.

[12]Peterson HD: Tangential excision. In Artz, C. P., Moncrief JA and Pruitt BA Jr (eds.): Burns: A Team Approach. Philadelphia, WB Saunders Company, 1979, p 235.

In the presence of chemical eye injury, patients will have a foreign body sensation and exhibit excessive tearing and blepharospasm. Should such an injury be suspected, initial treatment consists of prolonged water or saline irrigation. Epithelial defects are then identified by fluorescin stain, and, after thorough irrigation, a cycloplegic is administered to counteract the effects of the chemically-induced iritis (5). To maintain globe mobility, the eye is not patched. Temporary tarsorrhaphies or intermarginal sutures may be placed to provide corneal protection and promote epithelial healing in severe injuries (7). The tarsorrhaphy should be tied in a small bow over pieces of silastic tubing to facilitate daily examination of the globe. Chemical injuries about the eye may result in destruction of eyelid tissue and result in blindness if corneal perforation occurs because the globe cannot be protected.

Hospitalization. Curreri et al (1) and others (2) previously noted increased hospital stay in patients with chemical burns, as compared to patients with thermal injuries. This increased stay was attributed to slow wound healing following chemical skin injury (14-15). When hospital stays for patients sustaining WP burns, non-WP chemical burns, and thermal burns were compared to similar patients from Curreri's 1970 study (also from the US Army Institute of Surgical Research), the following were noted: (1) Regardless of the etiology of injury, hospital stays for burn patients are decreasing, which, in thermal injuries, has been attributed to earlier excision and grafting (16-17). (2) Patients with WP burns had the longest hospital stays in our study and this is in part secondary to the increased frequency of associated injuries and complications. (3) Patients sustaining non-WP chemical burns have shorter hospital stays than thermally injured patients with a burn of similar size.

---

[13]Silverstein P and Peterson HD: Treatment of eyelid deformities due to burns. Plast Reconstr Surg 51:38-43, 1973.

[14]Obermer E: Phosphorous burns. Lancet 1:202, 1943.

[15]Sinilo ML: Chemical burns and their treatment. Acta Chir Plast (Praha) 3:311-317, 1961.

[16]Burke JF, Bondoc CC, and Quinby WW: Primary burn excision and immediate grafting: a method shortening illness. J Trauma 14:389-395, 1974.

[17]Engrav LH, Heimbach DM, Reus JL, et al: Early excision and grafting vs. nonoperative treatment of burns of indeterminant depth: a randomized prospective study. J Trauma 23:1001-1004, 1983.

Specific Agents and Systemic Toxicity.

White Phosphorous. WP is commonly used as an incendiary in the manufacture of munitions. The military population is at increased risk of sustaining WP injuries, as our study confirms, with WP injuries occurring exclusively in military patients. The use of WP in the manufacture of certain fertilizers and fireworks also puts a select civilian population similarly at risk.

In the presence of air, WP is rapidly oxidized to phosphorous pentoxide (18). This exothermic reaction causes the phosphorous to burst into a yellow flame and give off dense white smoke with a characteristic garlic-like odor. The oxidation of phosphorous can be interrupted by eliminating the presence of oxygen; this is done by quenching the fire with water. When munitions containing WP explode, multiple particles may be imbedded in skin and soft tissue. These retained particles continue to smolder, and, even if doused with water initially, can reignite once the WP dries. Clothing often ignites, producing larger burns than those due solely to imbedded WP particles.

The initial treatment of WP burns consists of removal of all clothing. This is particularly important in hot climates or after an explosion, as the melting point of WP is 44° C and at temperatures greater than this, the liquid phase of WP may be present in clothing and difficult to identify. The skin must be irrigated with water to halt the ongoing oxidation, remove particles from the skin surface, and prevent reignition. The patient should be transported in saline or water-soaked dressings to prevent reignition of retained particles. The dressings must be kept moist until adequate debridement is accomplished.

Copper sulfate has been used to neutralize and aid in the identification of retained WP particles (1,5,19-21). Rinsing the wound with dilute (one percent or less) solutions of copper sulfate will cause a blue-black film of cupric

---

[18]Rabinowitch IM: Treatment of phosphorous burns. Canad Assoc J 48:291-296, 1943.

[19]Ben-Hur N, Giladi A, Applebaum J, et al: Phosphorous burns: the antidote: a new approach. Br J Plast Surg 25:245-249, 1972.

[20]Ben-Hur N, Neuman Z, Giladi A, et al: Phosphorous burns - a pathophysiological study. Br J Plast Surg 25:238-244, 1972.

[21]Ben-Hur N, Shani J, and Appelbaum J: Phosphorous burns in primates: a conclusive experimental study of a new specific therapy. Burns 4:246-253, 1978.

phosphide to form on the surface of the imbedded phosphorous, thus impeding oxidation and, more importantly, facilitating identification of imbedded particles to insure adequate debridement. Systemic absorption of copper sulfate may cause massive hemolysis and acute renal failure (1,22-23). This has been described in patients submerged in copper sulfate solutions or placed in dressings containing copper sulfate. The use of solutions with concentrations greater than one percent, even for lavage, has also been associated with these complications.

A safer method of detecting retained WP employs the use of the Wood's lamp (24). Phosphorous fluoresces under ultraviolet light, enhancing the recognition of imbedded particles. This method eliminates the potentially fatal toxicity seen with copper sulfate use and should be the preferred method of detection.

Absorption of phosphorous from the burn may occur and produce systemic toxicity. A few patients with sudden death from ventricular arrhythmias shortly after sustaining WP burns have been described (25). In an animal model, WP burns were shown to result in a reversal of the calcium-phosphorous ratio with electrocardiographic changes and an 85-percent mortality. At postmortem, pathologic changes of both the kidney and liver were noted (26). Though systemic toxicity from cutaneous phosphorous absorption is not well defined in man, the potential for its occurrence exists. In patients with extensive WP burns, serum electrolyte concentrations should be measured serially and the electrocardiogram monitored to detect potential systemic toxicity from phosphorous absorption.

---

[22]Mendolsen JA: Some principles of protection against burns from flame and incendiary munitions. J Trauma 11:286-294, 1971.

[23]Summerlin WT, Walder AI, and Moncrief JA: White phosphorous burns and massive hemolysis. J Trauma 7:476-484, 1967.

[24]Chemical burns and white phosphorous injury. In Emergency War Surgery. Washington, DC: US Government Printing Office, 1975.

[25]Bowen TE Jr, Whelan TJ Jr, and Nelson TG: Sudden death after phosphorous burns: experimental observations of hypocalcemia, hyperphosphatemia, and electrocardiographic abnormalities following production of a standard white phosphorous burn. Ann Surg 174:779-784, 1971.

[26]Appelbaum J, Ben-Hur N, and Shani J: Subcellular morphological changes in the rat kidney after phosphorous burn. Pathol Eur 10:145-154, 1975.

Phenol. Phenol and its derivatives are corrosive, aromatic, hydroxyl compounds with limited water solubility capable of producing rapid tissue destruction and fatal systemic toxicity (27). Coagulation necrosis occurs quickly, and penetration into tissues continues secondary to phenol's high lipid solubility. Dilution of phenol with water may increase tissue penetration by allowing the water solubilized phenol to penetrate the thick avascular eschar produced by contact with the more concentrated agent. Water irrigation is not uniformly recommended unless a high density shower is available (27). Phenol is more soluble in polyethylene glycol, and a polyethylene glycol wash will more effectively remove the phenol from the skin (1-2,27). Propylene glycol, glycerol, vegetable oil, and soap and water have also been employed, but are less effective than polyethylene glycol (27). A 50-percent solution of polyethylene glycol in water should be used because higher concentrations produce a significant exothermic reaction when the skin moisture further dilutes the glycol. If polyethylene glycol is not immediately available, intense water irrigation should be done followed by polyethylene glycol used as a second wash once it is obtained. Polyethylene glycol should also be removed from the skin surface by copious water irrigation because it too has been reported to produce systemic toxicity when absorbed through the burn wound (28).

Phenol is extremely toxic and may produce multisystem effects. With significant absorption, central nervous system depression, hyperthermia, hypotension, intravascular hemolysis, pulmonary edema, shock, and death will result, as occurred in our patient. Supportive measures are not specific and may include mechanical ventilation, invasive hemodynamic monitoring, and exchange transfusion. Obviating systemic absorption is most important in phenol burns and is done by washing the phenol from the skin as soon as possible. High density water lavage followed by a polyethylene glycol wash to remove the residual phenol is the preferred treatment.

Hydrocarbons. Cutaneous injury from immersion in gasoline and other hydrocarbons does occur and is often overlooked in victims of motor vehicle accidents who sustain prolonged exposure during extraction (1-2,5). The hydrocarbons' solvent properties promote cell membrane injury and dissolution of lipids, resulting in skin necrosis. Fortunately, most injuries are partial-thickness in character,

---

[27]Pardoe R, Minami RT, Sato RM, et al: Phenol burns. Burns 3:29-41, 1977.

[28]Bruns DE, Herold DA, Rodeheaver GT, et al: Polyethylene glycol intoxication in burn patients. Burns 9:49-52, 1982.

although full-thickness injuries can occur (29). Systemic toxicity, which often occurs after ingestion or inhalation (30), has been described from cutaneous absorption (10). Hydrocarbons are excreted through the lungs and absorption by any route may produce chemical pneumonitis and bronchitis. Vascular endothelial damage has been observed in the kidney and liver following gasoline inhalation and may produce glomerulonephritis and hepatitis (31). Cardiovascular complications, including sudden death, have been described following gasoline inhalation (32). Tetraethyl lead, an additive of gasoline, is absorbed through the skin and lead poisioning due to this has been described (10). Blood and urine lead levels should be monitored.

The treatment of hydrocarbon exposure consists of removal of all clothing and water irrigation as soon as possible. Early excision should be considered if lead toxicity occurs. In addition, lead poisoning from cutaneous absorption requires treatment with mercaprol, edetate calcium disodium, and penicillamine until urine and serum lead levels decrease (10).

Nitrates. The patient in our series burned by hot sodium nitrate solution sustained a 90.5 percent total body surface burn and died on the second postburn day. Though sodium nitrate does not produce cutaneous injury, systemic absorption of nitrates through a burn wound may result in methemoglobinemia (33). This diagnosis should be suspected in the cyanotic patient who is unresponsive to oxygen therapy and whose blood appears chocolate brown in color. Methemoglobin levels below 20 to 30 percent are usually asymptomatic and specific therapy is not required. Levels above 30 percent, with or without symptoms, should be treated by high flow oxygen and intravenous methylene blue administered slowly at a dose of one to two milligrams per kilogram body weight. Exchange transfusion may also benefit severe cases by rapidly decreasing circulating methemoglobin concentration and increasing the

---

[29] Hansbrough JF, Zapata-Sirvent R, Dominic W, et al: Hydrocarbon contact injuries. J Trauma 25:250-252, 1985.

[30] Rouse ET, Weese WC, and Kazemi H: Letter: gasoline ingestion. N Engl J Med 290:1092-1093, 1974.

[31] Beirne GJ and Brennan JT: Glomerulonephritis associated with hydrocarbon solvents: mediated by antiglomerular basement membrane antibody. Arch Environ Health 25:365-369, 1972.

[32] Bass M: Sudden sniffing death. JAMA 212:2075-2079, 1970.

[33] Harris JC, Rumack BH, Peterson RG, et al: Methemoglobinemia resulting from absorption of nitrates. JAMA 242:2869-2871, 1979.

oxygen-carrying capacity of the blood (34). Even though this chemical skin injury is rare, failure to diagnose and treat severe methemoglobinemia will result in death.

Formic Acid. Formic acid is a caustic organic acid, used in industry and agriculture, capable of causing full-thickness skin injury and systemic toxicity. Only five cases of formic acid skin burns have appeared previously in the medical literature (35-38). Our two patients had seven-percent and 17-percent partial-thickness skin injuries after being sprayed with a mixture of formic acid and hydrochloric acid. The patient with the 17-percent injury developed systemic toxicity manifested by acidosis, intravascular hemolysis, and hemoglobinuria. Both patients survived.

Formic and other acids cause cutaneous injury by coagulation necrosis. Systemic toxicity is common with ingestion (39) and is manifested by acidosis, hemolysis, and hemoglobinuria. Shock, renal failure, and pulmonary edema may occur. Systemic toxicity has been previouly described following cutaneous absorption in two patients (37-38). Hemolysis appears to be caused by the direct effect of formic acid on the red cells.

Initial management consists of aggressive wound lavage. Acidosis, if present, should be treated with intravenous bicarbonate. Minor hemolysis needs no treatment. Mannitol may be used to expand plasma volume and promote osmotic diuresis in the event of significant hemolysis. Exchange transfusion and hemodialysis have been used in formic acid poisoning (40) and may be required for patients in whom significant cutaneous absorption has occurred.

---

[34]Kirby NG: Sodium-nitrate poisoning treated by exchange transfusion. Lancet 1:594-595, 1955.

[35]Malizia E, Reale C, Pietropaoli P, et al: Formic acid intoxications. Acta Pharmacol Toxicol 41:342-347, 1977.

[36]Milbradt R: Zur Veratzung mit ameisensaurehaltigem Kalkloser. Berufsdermatosen 22:156-158, 1974.

[37]Ramstad KR, Reier K, and Skandsen S: Ets-Skader fororsaket av maursyre (silovaeska-konsentrat). Farmakoterapi 85, 1968 (in Norwegian).

[38]Sigurdsson J, Bjornsson A, and Gudmundsson ST: Formic acid burn - local and systemic effects: report of a case. Burns 9:358-361, 1983.

[39]Jefferys DB and Wiseman HM: Formic acid poisoning. Postgrad Med J 56:761-762, 1980.

[40]Naik RB, Stephens WP, Wilson DJ, et al: Ingestion of formic acid-containing agents - report of three fatal cases. Postgrad Med J 56:451-456, 1980.

DISCUSSION

Initial care of all chemical injuries should consist of removal of all clothing and water lavage, with special attention to adequate eye irrigation if chemical eye injury is suspected. In the special case of phenol, polyethylene glycol is the preferred irrigant and should be applied to the burn when available. Cutaneous absorption may occur in formate, hydrocarbon, nitrate, phenol, and WP burns and cause systemic toxicity. WP burns, typically war-related injuries, should be kept moist with water or saline dressings until all retained particles are removed to prevent ignition. Wood's lamp use to identify retained WP is preferred over copper sulfate irrigation. Inhalation of aerosolized chemicals may produce pulmonary injury and systemic toxicity, thus requiring accurate diagnosis and aggressive treatment. Later treatment of the burn wound, associated injuries, and complications was identical to that of thermally injured patients. The length of hospital stay for chemically injured patients has decreased with our current treatment regimen, which includes timely excision of the chemically burned tissues and immediate closure of the wound with cutaneous autografts.

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH COMPLETION REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: The Influence of Inhalation Injury and Pneumonia on Burn Mortality

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Khan Z. Shirani, MD, Lieutenant Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS: The Influence of Inhalation Injury and Pneumonia on Burn Mortality

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: Khan Z. Shirani, MD, Lieutenant Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

In order to assess the specific effects of inhalation injury and pneumonia on mortality in burn patients, we have reviewed the records of 1,058 patients treated at a single institution over a five-year period, 1980 through 1984. Within this study population, there were 373 patients (35 percent) who sustained inhalation injury diagnosed by bronchoscopy and/or ventilation perfusion lung scan. Of these, 141 (38 percent) subsequently developed pneumonia. Among patients without inhalation injury, pneumonia occurred in 60 of 685 (8.8 percent). A multiple logistic equation was developed to estimate expected mortality at any age and burn size for populations without either inhalation injury or pneumonia, with either alone, or with both. Subtraction of the expected mortality without either inhalation injury or pneumonia from the expected mortality in the presence of either or both permitted estimation of additional mortality produced by these complications. Inhalation injury alone increased mortality by a maximum of 20 percent and pneumonia by a maximum of 40 percent with a maximum increase in mortality of approximately 60 percent when both were present. The influence on mortality of these complications was maximal in the midrange of expected mortality for any age group. These data indicate that inhalation injury and pneumonia have significant, independent, additive effects on burn mortality and that these effects vary with age and burn size in a predictable manner.

BURNS
BURN MORTALITY PREDICTION
INHALATION INJURY
PNEUMONIA

THE INFLUENCE OF INHALATION INJURY AND PNEUMONIA ON BURN MORTALITY

INTRODUCTION

It is generally recognized that pulmonary complications adversely affect the outcome of patients with burn injury. In an autopsy study, 70 percent of all fire victims who died within 12 hours of burns had evidence of inhalation injury (1), indicating that toxic gases and products of incomplete combustion contribute significantly to early postburn death. Cutaneous burns activate the complement cascade and induce intrapulmonary leukocyte aggregation, release of free radicals of oxygen, and pulmonary damage, possibly adding further respiratory insult to patients with inhalation injury. In addition, global immunosuppression accompanies and is proportional to the extent of burn injury. As a consequence, respiratory tract infection is the most common complication of burns (2). Although both inhalation injury and pneumonia reduce patient survival, the specific contributions of these complications to age and burn size-dependent patient mortality have not been completely determined. The present study attempts to define, in explicit terms, the contributions of inhalation injury and pneumonia to patient mortality.

MATERIALS AND METHODS

The records of 1,058 consecutive burn patients treated at this Institute during the five-year period between January 1980 and December 1984 were reviewed. The records were complete for all patients. Data were gathered on the status of inhalation injury on admission and on development of pneumonia during hospitalization. Patient survival was also noted. All patients received uniform care. Fluid resuscitation was according to a modified Brooke formula (3). Burn wounds were managed with applications of silver sulfadiazine (4) and mafanide acetate cream alternated every 12 hours (5).

---

[1] Zikria BA, Weston GC, Chodoff M, et al: Smoke and carbon monoxide poisoning in fire victims. J Trauma 12:642-645, 1972.

[2] Pruitt BA Jr, Flemma RJ, DiVincenti FC, et al: Pulmonary complications in burn patients. J Thorac Cardiovascu Surg 59:7-20, 1970.

[3] Pruitt BA Jr: Fluid resuscitation for extensively burned patients. J Trauma 21:690-692, 1981.

[4] Fox CL Jr: Silver sulfadiazine, a new topical therapy for Pseudomonas in burns. Arch Surg 96:184-188, 1968.

[5] Lindbergh RR, Moncrief JA, Switzer WE, et al: The successful control of burn wound sepsis. J Trauma 5:601-616, 1965.

Nutritional support was provided to meet increased metabolic demands. Excision and grafting of the burn wound usually began during the first postburn week.

Inhalation injury was suspected in patients with facial burns and patients involved in structural fires or burned in a closed space. Patients were also considered at risk if they were mentally or physically impaired at the time of the accident. All patients at risk were investigated by bronchoscopy, ^{133}Xenon lung scan, or both (6). Bronchoscopic diagnosis of inhalation injury in these individuals rested on demonstration of inflammatory changes in the respiratory tract. These changes included mucosal erythema, edema, or ulceration and submucosal hemorrhages with or without carbon deposition in the tracheobronchial tree (7). Patients with a positive ^{133}Xenon lung scan independent of bronchoscopic findings were also considered to have inhalation injury. An abnormal lung scan consisted of ventilation perfusion mismatch or isotope retention exceeding 90 seconds (8).

Of the total study population, 487 patients were considered at risk for inhalation injury and were further studied. Bronchoscopy alone was performed in 140 patients, ^{133}Xenon lung scan alone in 106 patients, and both procedures in 241 patients. In patients with purulent sputum and physical findings suggestive of pneumonia, a chest roentgenogram was obtained and sputum or endotracheal secretions were examined by Gram-stained smears and by culture. Patients exhibiting clinical signs and symptoms of respiratory tract infection and characteristic pneumonic infiltrates on chest roentgenograms received antibiotics. Initial antibiotic therapy for pneumonia was modified, if needed, when final culture and sensitivity results became available (9).

Statistical differences between groups of patients with and without inhalation injury were assessed using "Student's" t test. Multiple logistic regression technique was used to develop a predictor of the occurrence of inhalation injury. A basic age and burn size-specific index, based on our experience in the treatment of over 6,000 burn patients at this Institute

---

[6]DiVincenti FC, Pruitt BA Jr, Reckler JM: Inhalation injuries. J Trauma 11:109-117, 1971.

[7]Hunt JL, Agee RN, Pruitt BA Jr: Fiberoptic bronchoscopy in acute inhalation injury. J Trauma 15:641-649, 1975.

[8]Moylan JA Jr, Wilmore DW, Mouton DE, et al: Early diagnosis of inhalation injury using ^{133}Xenon lung scan. Ann Surg 176:477-484, 1972.

[9]Shirani KZ, McManus AT, Vaughan GM, et al: Effects of environment on infection in burn patients. Arch Surg 121:31-36, 1986.

over the past three decades, was used to assess severity of injury. In addition, the present cohort of 1,058 patients was analyzed separately to assess specific contributions of inhalation injury and pneumonia to mortality.

RESULTS

A diagnosis of inhalation injury was made in 373 patients, 35.3 percent of the entire cohort. Pertinent demographic data on the study patients are shown in Figure 1 and Table 1. Twenty-one percent of the patients without inhalation injury and 73 percent of those with inhalation injury were confined in a closed environment at the time of their burns. Respectively, 93 percent and 52 percent of the patients with and without inhalation injury sustained facial burns. Predicted mortality, based on the severity index, and observed mortality, respectively, were 17.9 percent and 9.6 percent in patients without inhalation injury and 48.2 percent and 46.6 percent in patients with inhalation injury. The relationship between inhalation injury and burn size for the entire population is shown in Figure 2. With increasing burn size, there was a corresponding rise in the incidence of inhalation injury. With increasing burn size, the incidence of pneumonia increased in patients with injuries smaller than 90 percent of the total body surface; beyond this, the incidence decreased, possibly because many such severely injured patients died soon after injury (Figure 3). The incidence of pneumonia and predicted and observed mortality with or without inhalation injury, diagnosed either by bronchoscopy or by ^{133}Xenon lung scan alone, are displayed in Figure 4. There was a stepwise increase in the incidence of pneumonia from the patient group without inhalation injury (8.8 percent) to the group with positive ^{133}Xenon lung scan only (19.5 percent) to the group with positive bronchoscopy (45.8 percent). Figure 5 displays the incidence of pneumonia, postburn day of diagnosis of pneumonia, age, and burn size for the entire cohort and for patients with and without inhalation injury diagnosed either by bronchoscopy or by ^{133}Xenon lung scan alone. The average day of diagnosis of pneumonia was similar in all groups, 11 days for the entire cohort, 15 days for patients without inhalation injury, and 10 days and 11 days, respectively, for patients with inhalation injury diagnosed by bronchoscopy or ^{133}Xenon lung scan. It should be noted, however, that in patients without inhalation injury, only 39 percent of the cases of pneumonia developed within the first week, while in those with inhalation injury, 69 percent developed pneumonia in that interval. The times to pneumonia for bronchoscopy-positive and ^{133}Xenon lung scan-positive patients were similar and are shown in Figure 6. The average age of all patients with pneumonia was 45 years and the average burn size was greater than 52 percent of the total body surface area (Figure 5).

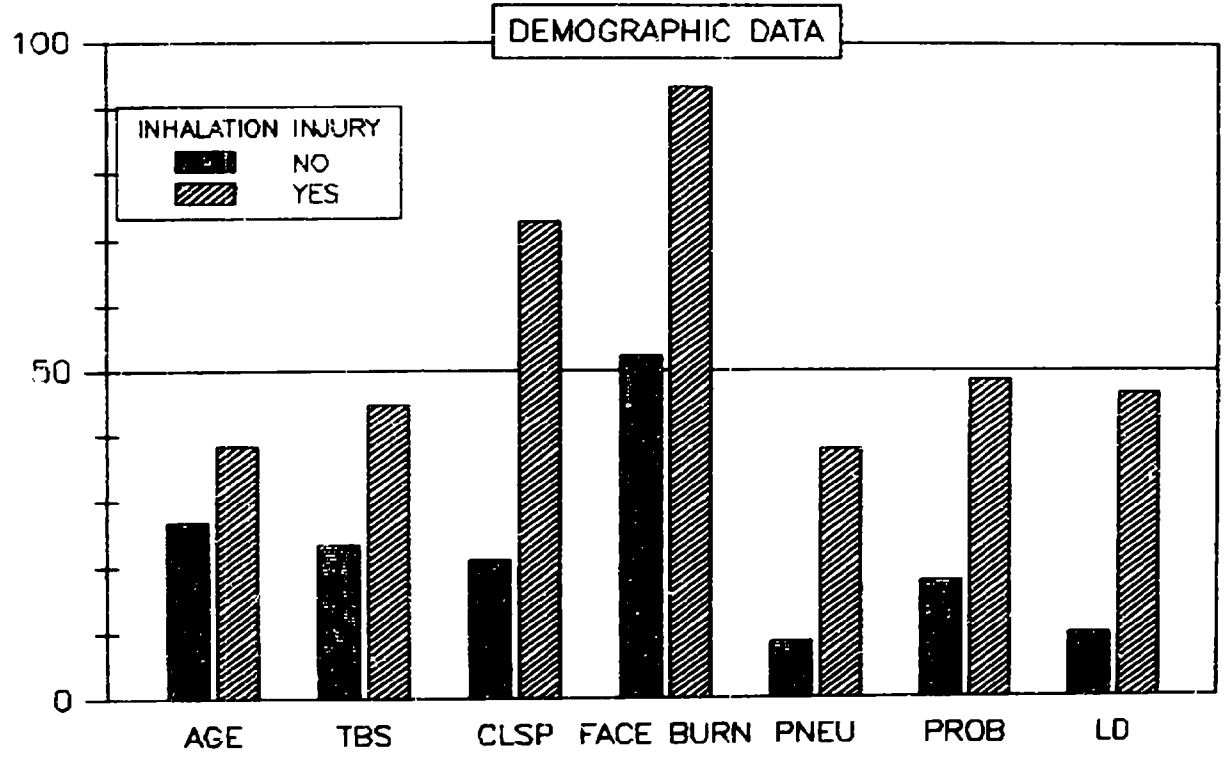


FIGURE 1. Data on patients with and without inhalation injury. In patients with inhalation injury, age and total burn size (TBS) were slightly higher; more patients sustained injury in closed spaces (CLSP) and there was a higher incidence of facial burns and pneumonia (PNEU). Age and burn size-adjusted probability of death (PROB) and observed mortality (LD) were also higher in that group.

TABLE 1. Patient Characteristics

	(n)	Age in Years	Burn Size Percentage	Percentage of Patients with Closed Space Injury	Percentage of Patients with Face Burn	Probability of Death	Observed Mortality
No Inhalation Injury	685	27(20)	23(19)	21	52	17.9%	9.6%
Positive Xenography	113	37(18)	33(22)	61	89	31.2%	21.2%
Positive Bronchoscopy	260	39(21)	50(24)	78	95	55.6%	57.7%

( ) = Standard Deviation

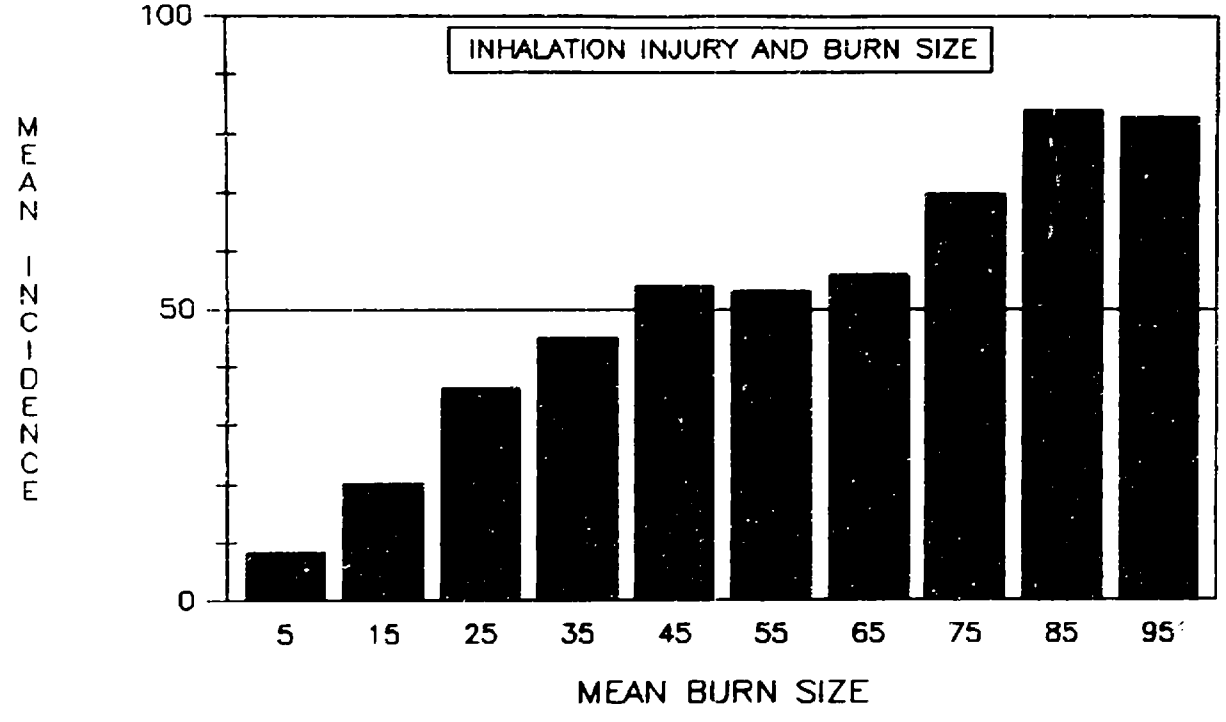


FIGURE 2. Relationship between burn size and incidence of inhalation injury illustrates the rise in occurrence of inhalation injury with increasing burn size.

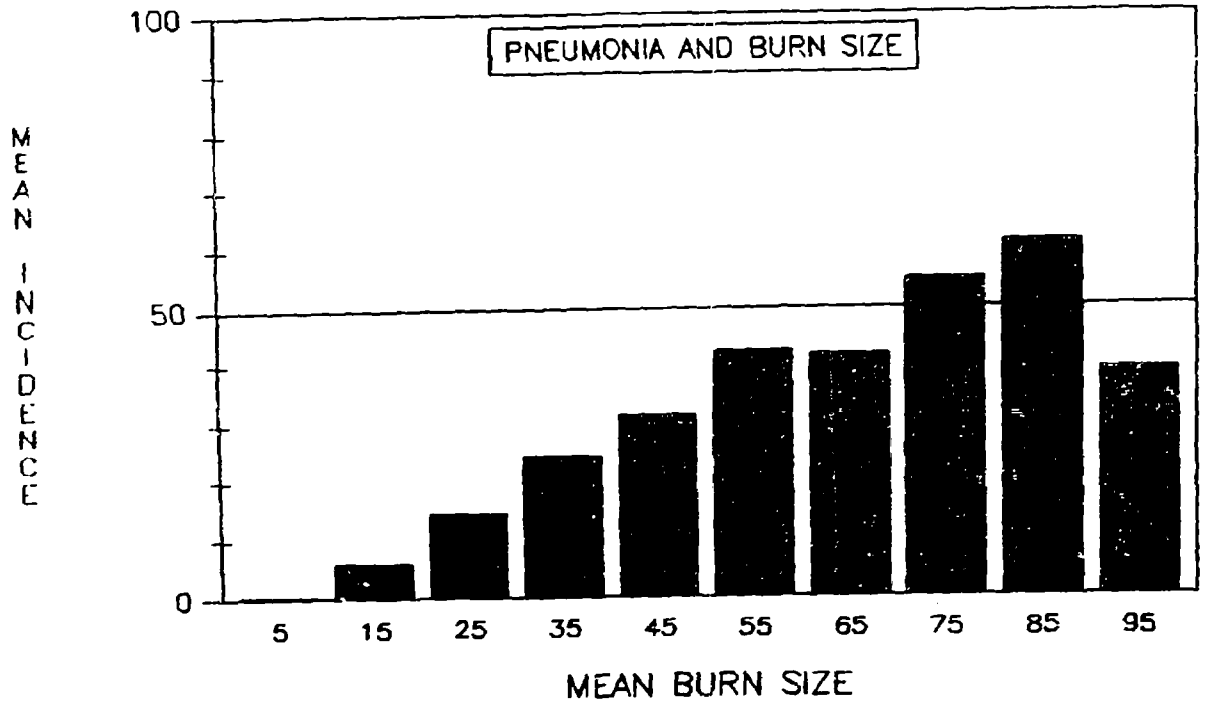


FIGURE 3. There was a progressive rise in the incidence of pneumonia with increasing burn size except in patients with burns greater than 85 percent of the total body surface.

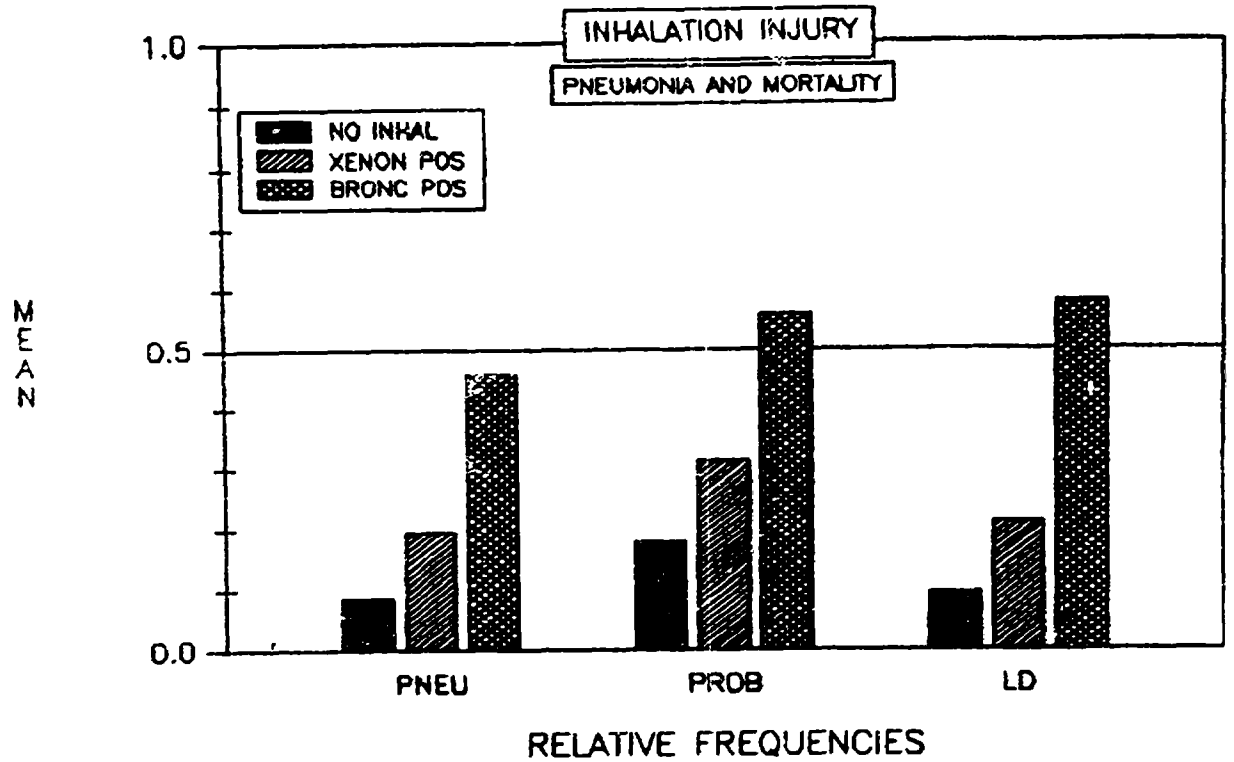


FIGURE 4. There was a stepwise rise in the occurrence of pneumonia (PNEU) from patients without inhalation injury (NO INHAL) to those having inhalation injury diagnosed either by ^{133}Xenon lung scan alone (XENON POS) or by bronchoscopy (BRONC POS). Age and burn size-adjusted expected mortality (PROB) and observed mortality (LD) for patients with inhalation injury (XENON POS, BRONC POS) were also higher.

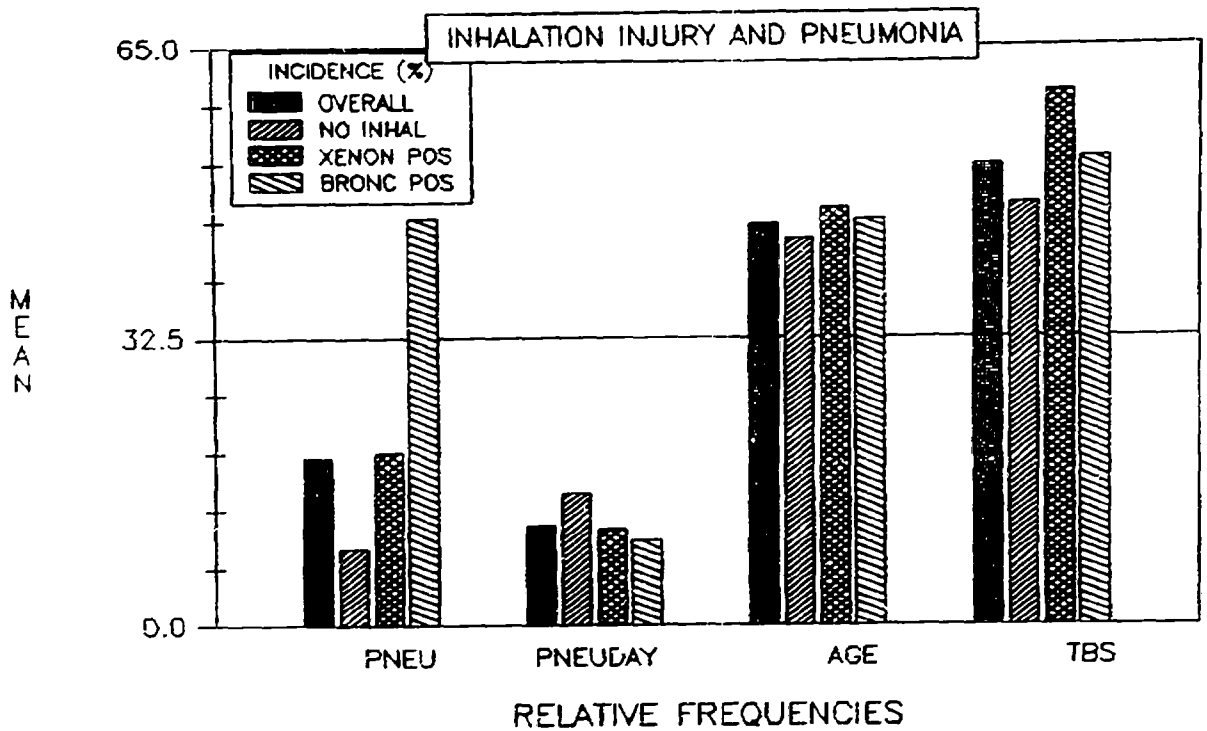


FIGURE 5. Overall incidence of pneumonia (PNEU), day of diagnosis of pneumonia (PNEUDAY), age, and total burn size (TBS) for entire cohort (OVERALL) for patients without inhalation injury (NO INHAL) and for patients with inhalation injury diagnosed either by ^{133}Xenon lung scan only (XENON POS) or by bronchoscopy (BRONC POS) are shown. Note the highest incidence of pneumonia in patients with inhalation injury diagnosed by bronchoscopy. The average day of diagnosis of pneumonia, age, and burn size, however, appear similar for all groups.

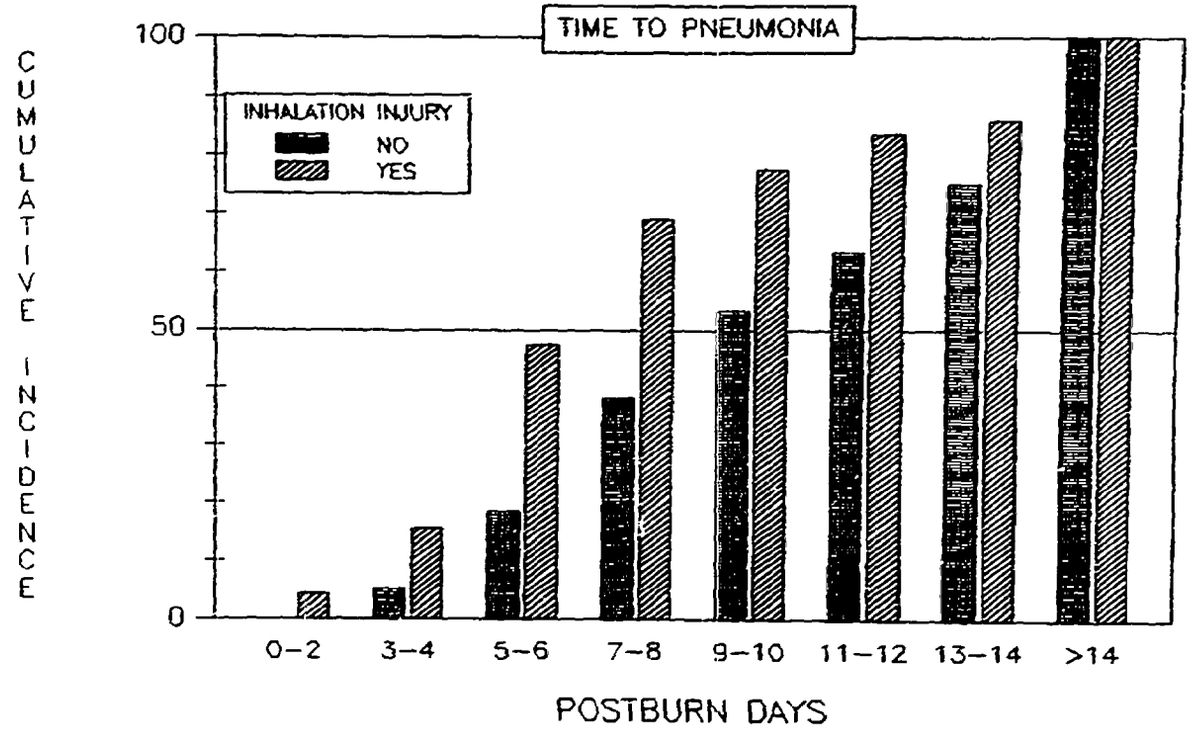


FIGURE 6. Times to pneumonia for patients with inhalation injury diagnosed by ^{133}Xenon lung scan only (XENON POS) or by bronchoscopy (BRONC POS) were similar. Early pneumonia was more frequent in patients with inhalation injury.

Using a stepwise logistic regression algorithm, a predictor of the occurrence of inhalation injury was developed. The variables entering the equation were injury in a closed space (CLSP), presence of facial burns (FB), age, and total burn size (TBS):

$$y = -4.4165 + 1.61(CLSP) + 1.77(FB) + .0237(TBS) + .0268(AGE)$$

CLSP = 0,1 (Absence, Presence of Injury in Closed Space)

FB = 0,1 (Absence, Presence of Facial Burn)

TBS = Total Burn as Percent of Total Body Surface Area

AGE = Age in Years

$$P_{(II)} = \frac{e^{Y}}{1 + e^{Y}}$$

$P_{(II)}$ = Expected Proportion of Such Patients with Inhalation Injury (Limits = 0,1)

A comparison of the observed incidence of inhalation injury and that predicted by the above equation is shown in Figure 7. At each level, prediction closely approximated observation.

Multiple logistic regression was also employed in the analysis of mortality of the entire cohort as a function of total burn size (TBS), age, inhalation injury (II), and pneumonia (PNEU), yielding the following equation:

$$y = -3.4953 + .09589(TBS) - .19881(AGE) + .0044788(AGE^2) - .000020314(AGE^3) + .59056(II) + .92530(PNEU)$$

TBS = Total Burn as Percent of Total Body Surface Area

AGE = Age in Years

II = -1,+1 (Absence, Presence of Inhalation Injury)

PNEU = -1,+1 (Absence, Presence of Pneumonia)

$$P = \frac{e^{Y}}{1 + e^{Y}}$$

P = Expected Mortality (Limits = 0,1)

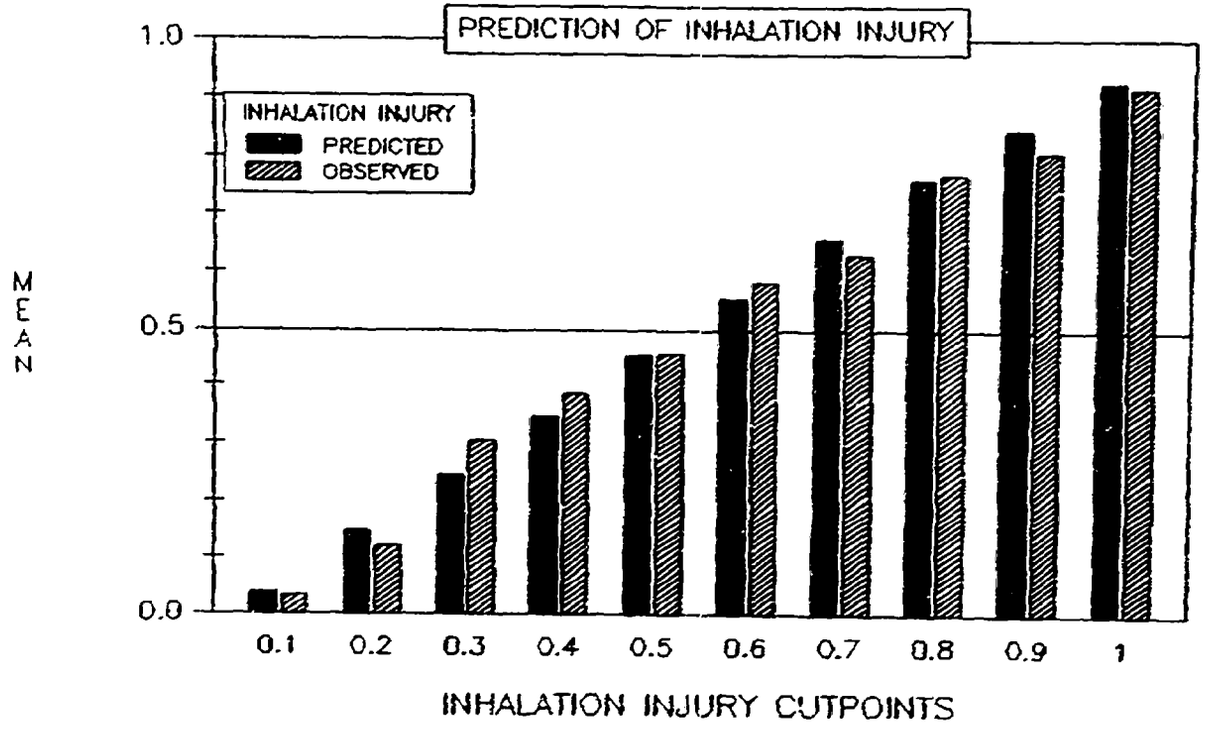


FIGURE 7. Observed and predicted incidences of inhalation injury (INH INJ) were remarkably similar (see text for details).

This equation was used to estimate expected mortality in the absence of both inhalation injury and pneumonia, in the presence of either alone, and in the presence of both. Subtraction of the mortality expected without inhalation injury or pneumonia from that expected in the presence of those complications permitted the construction of three-dimensional graphs depicting specific contributions to mortality of inhalation injury alone (Figure 8), pneumonia alone (Figure 9), and inhalation injury and pneumonia combined (Figure 10). Expected mortality increased by a maximum of 20 percent in the presence of inhalation injury alone, 40 percent in the presence of pneumonia alone, and 60 percent when both inhalation injury and pneumonia were present. The contributions of inhalation injury and pneumonia were found to be independent and additive. Expected mortality in patients with very small or very large burns appeared to be relatively uninfluenced by these pulmonary complications except at the extremes of age.

DISCUSSION

In the present study, we have sought to determine how inhalation injury and pneumonia influence the outcome after burn injury. In this consecutive series, we confirm previous findings in a smaller sample of patients in whom inhalation injury appeared to increase mortality (10). Additionally, the present study estimates, in a quantitative manner, the increase in patient mortality that attends inhalation injury. Burn patient survival is also reduced in the presence of pulmonary infection (11).

The present study estimates the precise roles of each of these pulmonary complications in the outcome of patients with burns. Our data indicate that both inhalation injury and pneumonia are detrimental to patient survival and that a maximal deleterious effect occurs in the midrange of burn severity. We have attempted to depict the explicit, additive changes in mortality attributable to these pulmonary complications as they relate to age and total burn size. The age and burn size-specific mortality increased by a maximum of 20 percent in the presence of inhalation injury alone, by 40 percent in the presence of pneumonia alone, and by 60 percent in the presence of both.

Inhalation injury and pneumonia contributed minimally to mortality in patients with small burns who did well despite

---

[10] Agee RN, Long JM III, Hunt JL, et al: Use of ^{133}Xenon in early diagnosis of inhalation injury. J Trauma 16:218-224, 1976.

[11] Dowell AR, Kilburn KH, Pratt PC: Short-term exposure to nitrogen dioxide. Arch Intern Med 128:74-80, 1971.

INCREASE IN MORTALITY

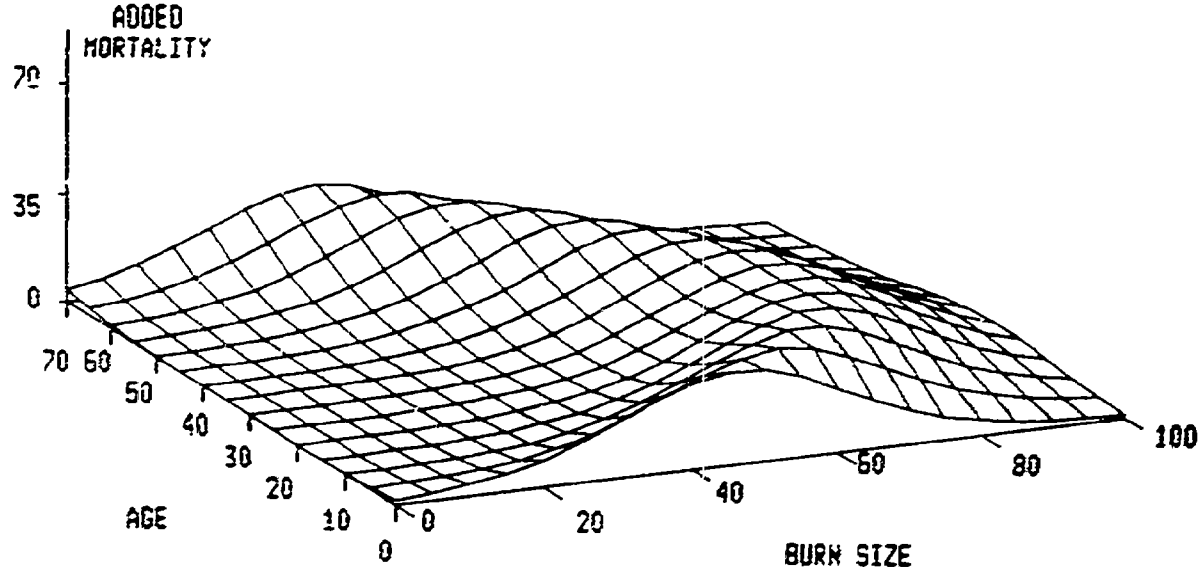


FIGURE 8. Burn size as percent of total body surface area on X axis, age on Y axis, and increment in mortality due to the presence of inhalation injury on Z axis are shown. Mortality, in the presence of inhalation injury alone, rose by a maximum of approximately 20 percent in patients in midrange of severity of injury as indexed by age and burn size.

INCREASE IN MORTALITY

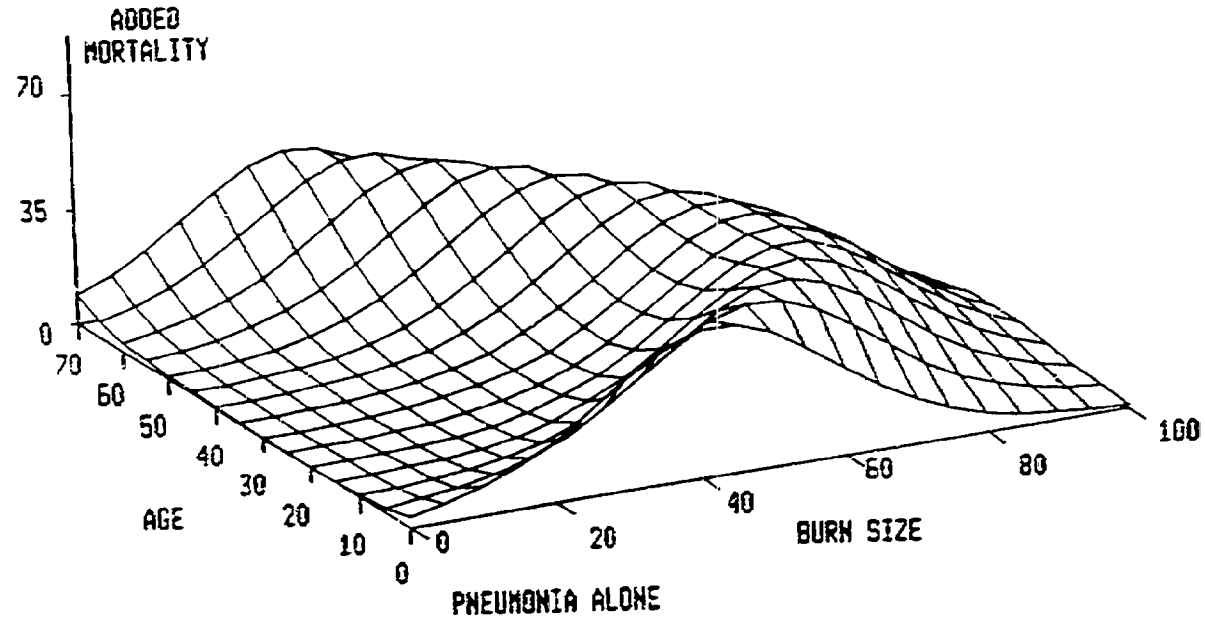


FIGURE 9. Burn size as percent of total body surface area on X axis, age on Y axis, and increment in mortality due to the presence of pneumonia on Z axis are shown. Mortality, in the presence of pneumonia alone, rose by a maximum of approximately 40 percent in patients in midrange of age and burn size.

INCREASE IN MORTALITY

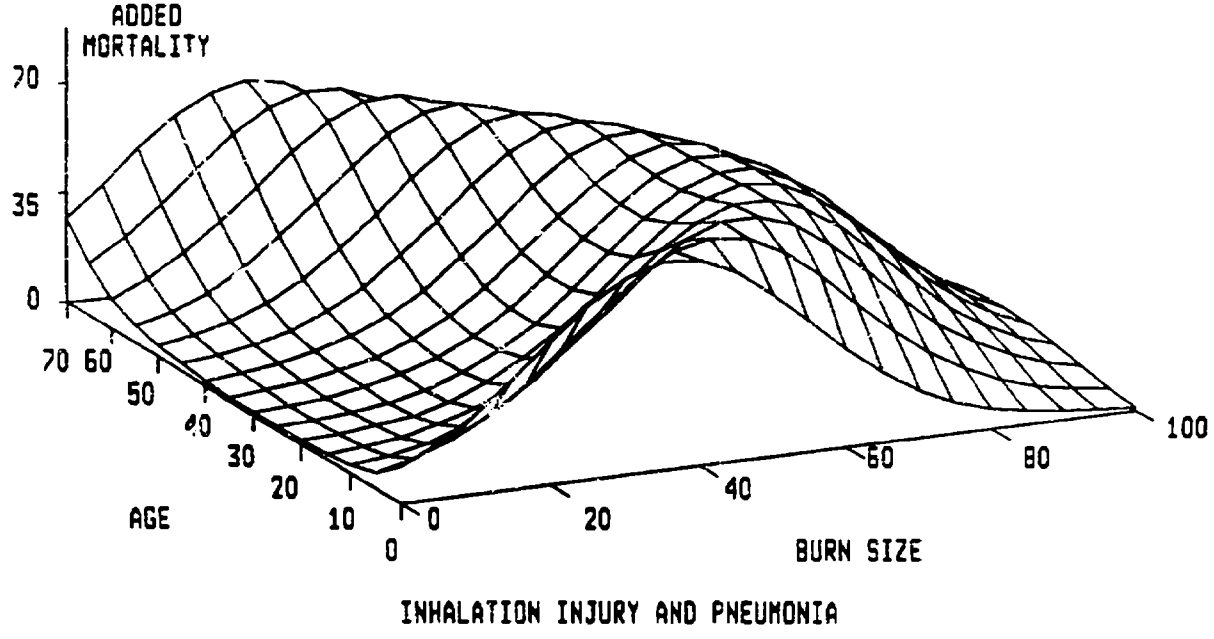


FIGURE 10. Burn size as percent of total body surface area on X axis, age on Y axis, and increment in mortality due to the presence of inhalation injury on Z axis are shown. Mortality rose by a maximum of approximately 60 percent in patients in midrange of age and burn size when both inhalation injury and pneumonia were present.

these ills. Similarly, in patients with extensive burns, in whom the traumatic insult exceeded the physiologic reserve, neither the presence nor the absence of pulmonary complications altered the dismal outcome.

Interestingly enough, despite similar age and burn size, the incidence of pneumonia in patients with positive bronchoscopic examination was higher than that in patients having either a positive ^{133}Xenon lung scan alone or no inhalation injury. It appears that respiratory tract damage that lends itself to diagnosis by lung scan alone, in the absence of positive bronchoscopic findings, represents a less severe form of injury than that detectable by bronchoscopy. The increased incidence of pneumonia in patients with visually demonstrable tracheobronchial inflammation appears to be ascribable to more extensive tracheobronchial injury. With massive tissue necrosis and disruption of the alveolar capillary membrane (11-12), protein-rich plasma exudes into the tracheobronchial tree and may serve as a medium for bacterial growth. Inhalation injury, in addition to inflicting structural damage on the respiratory tract epithelium, impairs surfactant production (13) and mucocilliary transport and produces atelectasis (14). Inhalation injury also impairs pulmonary macrophage function (12). The net result of these pulmonary changes, combined with the global immunosupression (15) of burns, is the development of respiratory tract infection. In patients with inhalation injury, early pneumonia was more common than in those without inhalation injury; in patients with inhalation injury, 69 percent of pneumonias occurred within the first postburn week while the corresponding figure was 38 percent for patients without inhalation injury.

In individuals with positive bronchoscopy, the ^{133}Xenon lung scan added little to either diagnosis or prognosis. Routine use of ^{133}Xenon lung scan in patients with positive bronchoscopic examinations seems, therefore, unwarranted. A positive ^{133}Xenon lung scan in a patient with negative bronchoscopy, on the other hand, assumes prognostic

---

[12]Sherwin RP and Richters V: Lung capillary permeability. Arch Intern Med 128:61-68, 1971.

[13]Nieman GF, Clark WR, Wax SD, et al: The effect of smoke inhalation on pulmonary surfactant. Ann Surg 191:171-181, 1980.

[14]Loke J, Paul E, Virgulto JA, et al: Rabbit lung after acute smoke inhalation: cellular responses and scanning electron microscopy. Arch Surg 119:956-959, 1984.

[15]Miller CL and Baker CC: Changes in lymphocyte activity after thermal injury: the role of suppressor cells. J Clin Invest 63:202-210, 1979.

significance, since the incidence of pneumonia is higher in such patients than in those having no inhalation injury. Thus far, a precise grading of clinical pulmonary damage consequent to inhalation injury has been impossible. In this regard, the present data allow partitioning of patients with inhalation injury into two broad categories, those with positive bronchoscopy irrespective of the status of the ^{133}Xenon lung scan and those with positive ^{133}Xenon lung scan only. Such classification is pragmatic and permits sorting of burn patients into those without inhalation injury, those with modest inhalation injury (^{133}Xenon lung scan-positive only), and those with severe inhalation injury (bronchoscopy-positive). Such stratification of pulmonary damage may permit further refinement of estimates of risk.

A predictor of the proportional frequency of occurrence of inhalation injury was developed which takes into account immediately available information such as injury in a closed space, facial burns, age, and total burn size. Such information should be useful in triage.

In summary, our data indicate that both inhalation injury and pneumonia exert discrete, measurable effects on patient mortality and can, therefore, be used along with age and total burn size to predict the likelihood of death in burn patients with such complications. Inhalation injury diagnosed on the basis of bronchoscopic findings carries a graver prognosis than pulmonary damage detectable only by ^{133}Xenon lung scan. Both complications add to the total physiologic insult in a predictable manner, having little effect on mortality in patients with small injuries where the total burn can be borne or in patients with very large injuries whose physiologic capacity is exceeded by the injury alone. In the central severity groups, however, the complications may make a sublethal injury lethal. It is in these groups that better management of pulmonary complications may improve survival.

PRESENTATIONS/PUBLICATIONS

Shirani KZ: The Influence of Inhalation Injury and Pneumonia on Burn Mortality. Presented at the 18th Annual Meeting of the American Burn Association, Chicago, Illinois, 11 April 1986.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DAOG6971	2. DATE OF SUMMARY: 86 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 85 10 01	4. KIND OF SUMMARY: D	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DISB'N INSTR'N: CX	9. LEVEL OF SUM A. WORK UNIT:

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AD	162
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY87-01		

11. TITLE *(Precede with Security Classification Code)*
(U) Evaluation of Burn Wound Care in Troops with Burn Injury

12. SUBJECT AREAS
06 05 Clinical Medicine 06 13 Microbiology 06 15 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
76 10	CONT	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY: Brad A. Pruitt

a. DATE EFFECTIVE	b. CONTRACT/GRANT NUMBER	c. TYPE	d. AMOUNT	e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS *(In thousands)*
86	1.5	95
87	1.5	98

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: MC MANUS, W F
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-3301
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR *(If available)* g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burn Injury; (U) Topical Therapy; (U) Sulfamylon; (U) 5% Sulfamylon Acetate Solution; (U) Volunteers;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

22. (Continued) (U) Autografts; (U) RAII

23. (U) The cause of infection in the wounds of burn patients has continued to be a major area of study in order to improve the survival of the severely burned patient. Such studies have included the use of five-percent aqueous Sulfamylon soaks, the effects of burn wound excision on survival and function, and the effectiveness of skin substitutes.

24. (U) Patients admitted to this Institute for care following thermal, chemical, or electric injury may be, depending on the specific injury, included in studies of these newer modalities of care.

25. (U) 8501 - 8512. One hundred fifty-nine patients were treated with five-percent aqueous Sulfamylon soaks during the period of this report. Twenty of these 159 patients exhibited mild cutaneous atopy. This low incidence of mild side effects of five-percent aqueous Sulfamylon and its continued clinical effectiveness speak for the continued use of this valuable therapeutic agent.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE ☆U.S. G.P.O. 1984-421-646/17001

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: E-Z Derm™ and Biobrane® - A Comparative Study

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Stephen M. Pratt, MD
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: E-Z Derm™ and Biobrane® - A Comparative Study

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: Stephen M. Pratt, MD
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Synthetic dressings are useful in the treatment of excised burn wounds when immediate autografting cannot be performed. A controlled prospective trial comparing E-Z Derm™ and Biobrane® was conducted on paired excised burn wounds prior to autografting. Each material was evaluated using a wound dressing index which numerically rates each of five factors, i.e., adherence, conformation, pliability, granulation tissue formation, and suppuration. With respect to suppuration beneath the dressing, E-Z Derm™ proved superior to Biobrane®. A superior bed of graftable fibrovascular tissue in terms of graftability was achieved with E-Z Derm™ as compared to Biobrane®, with total graft "take" after dressing removal of 93 and 80 percent, respectively. No statistical differences were noted in the remaining three categories.

A consistent and objective means for evaluating biologic dressings is presented and used to assess E-Z Derm™ and Biobrane® as temporary wound covers on excised burn wounds prior to autografting. In this context, both materials demonstrate efficacy, although E-Z Derm™ proved better in two of five categories.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: E-Z Derm™ and Biobrane® - A Comparative Study

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Stephen M. Pratt, MD
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

REPORT CONTROL SYMBOL - MEDDH-288(R1)

UNCLASSIFIED

97 - A

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: E-Z Derm™ and Biobrane® - A Comparative Study

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: Stephen M. Pratt, MD
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

REPORT CONTROL SYMBOL: MEDDH-288(R1)

Synthetic dressings are useful in the treatment of excised burn wounds when immediate autografting cannot be performed. A controlled prospective trial comparing E-Z Derm™ and Biobrane® was conducted on paired excised burn wounds prior to autografting. Each material was evaluated using a wound dressing index which numerically rates each of five factors, i.e., adherence, conformation, pliability, granulation tissue formation, and suppuration. With respect to suppuration beneath the dressing, E-Z Derm™ proved superior to Biobrane®. A superior bed of graftable fibrovascular tissue in terms of graftability was achieved with E-Z Derm™ as compared to Biobrane®, with total graft "take" after dressing removal of 93 and 80 percent, respectively. No statistical differences were noted in the remaining three categories.

A consistent and objective means for evaluating biologic dressings is presented and used to assess E-Z Derm™ and Biobrane® as temporary wound covers on excised burn wounds prior to autografting. In this context, both materials demonstrate efficacy, although E-Z Derm™ proved better in two of five categories.

E-Z Derm™ AND Biobrane® - A COMPARATIVE STUDY

INTRODUCTION

Closure of the burn wound is sine qua non to the recovery of the thermally injured patient. Because lack of available donor sites can delay wound closure, alternatives to autografting have been advanced as temporizing measures. Autograft has been widely recognized as the "gold standard" for temporary wound closure since its use in burns was first advanced by Pollack in 1870 (1). Control of water and protein loss from the wound, support of the production of a graftable fibrovascular bed, control of microbial proliferation, and pain control are recognized benefits of allograft use (2-4). Additionally, increased survival with burn wound excision and coverage has been documented (3); however, allograft availability, eventual rejection, and storage requirements have imposed limitations on its use. For this reason, a great deal of interest has been generated in the development of biologic and synthetic materials to serve as skin substitutes. Biobrane®, a collagen-synthetic bilaminar membrane, has been documented to be efficacious when compared with porcine xenograft and human allograft (5). Recently, E-Z Derm™, an aldehyde-treated xenograft, has been introduced, and reportedly shares the properties of xenograft with the added advantages of room temperature storage, longer wound retention times, and decreased antigenicity (6-7). A controlled prospective

---

[1]Freshwater MF and Krizek TJ: Skin grafting of burns: a centennial. J Trauma 11:862-865, 1971.

[2]Levine NS, Salisbury RE, and Mason AD Jr: The effect of early surgical excision and homografting on survival of burned rats and of intraperitoneally-infected burned rats. Plast Reconstr Surg 56:423-429, 1975.

[3]Miller TA, Switzer WE, Foley FD, et al: Early homografting of second degree burns. Plast Reconstr Surg 40:117-125, 1967.

[4]Pruitt BA Jr and Levine NS: Characteristics and use of biological dressings and skin substitutes. Arch Surg 119:312-322, 1984.

[5]Tavis MJ, Thornton JW, Butlett RH, et al: A new composite skin prothesis. Burns 7:123-130, 1980.

[6]Piccolo NA, Petro JA, and Salisbury RE: Increased adherence time of porcine xenograft pretreated with a cross-linking agent. Proceedings of the American Burn Association (Abstract 44):51-52, 1983.

[7]Schechter I: Prolonged retention of glutaraldehyde-treated skin allografts and xenografts: immunological and histological studies. Ann Surg 182:699-704, 1975.

comparative trial of these two dressings on excised burn wounds prior to autografting was conducted. Utilizing a previously described wound dressing index to quantify differences in each of five categories (8), meaningful data can be obtained to facilitate efficacy determinations; comparisons between the various biologic dressings and skin substitutes can be achieved.

MATERIALS AND METHODS

Fourteen sites were compared in 10 patients with a mean age of 33.6 years and a mean total body surface area burn size of 58 percent. Sites were comparable with respect to anatomic location, method and depth of excision, and size. In all cases, tangential excision (11 sites) and fascial excision (three sites) were followed by immediate dressing placement. E-Z Derm™ was applied in six-centimeter wide strips, with either side placed against the wound surface. Biobrane® of appropriate size was expanded until taunt and stapled into place. All excesses were trimmed as necessary. On extremities, both wound dressings were wrapped with a light gauze dressing for 24 hours to prevent early mechanical disruption, following which the wound dressings were exposed. Daily observations were recorded by the patient's primary physician, with particular attention given to adherence, conformation to the wound surface, and dressing pliability. The wound bed was also assessed for the presence of suppuration and granulation tissue formation. Large areas of nonadherence or suppuration beneath the dressing necessitated removal, culture, and treatment of the site with topical agents (sulfamylon and Silvadene® alternated every two hours). Small areas were locally excised and treated topically until reapplication of either dressing was clinically indicated. When adequate donor sites were available and the patient's condition permitted, dressing removal and autografting were performed.

Dressings were compared with respect to five properties relating to the dressing and wound site (Table 1). In addition, final autograft "take" on each site was noted.

The Wilcoxon matched-pair signed-rank's test was used to test against the null hypothesis that no difference exists between the two test materials in each category assessed.

---

[8]Roberts LW, McManus WF, Shirani KZ, et al: Biobrane® and porcine for the excised wound: a comparative study. J Trauma (in press).

TABLE 1

DEFINITION OF GRADING SCALES

FORMATION OF GRANULATION TISSUE

1 = None
2 = Scanty with irregular distribution and wound debris
3 = Irregular surface with wound debris or superficial exudate
4 = Clean granulating surface with minimal fibrosis or debris
5 = Beefy red, uniformly smooth, clean

PRESENCE OF SUPPURATION

1 = Frankly purulent collection beneath dressing
2 = Moderate seropurulent collection beneath dressing
3 = Slight seropurulent collection beneath dressing
4 = Slight serous collection beneath dressing
5 = None

ADHERENCE TO TEST DRESSING

1 = Slides off test site spontaneously
2 = Easily dislodged
3 = Easily removed with forceps
4 = Strips off with resistance
5 = Strips off with resistance and brisk bleeding

CONFORMATION TO WOUND SURFACE

1 = Buckled, wrinkled, poor wound contact
2 = Conforms only to flat wound surface
3 = Torsion and tension must be applied to gain maximal wound surface contact with minimal wrinkling present
4 = Torsion and tension must be applied to gain maximal wound surface contact with no wrinkling present
5 = Mimics skin

PLIABILITY OF TEST DRESSING

1 = Stiff prior to wound application
2 = Loses pliability unrelated to recipient test site condition
3 = Loses pliability related to absorption of wound exudate
4 = Maintains most of its pliability despite absorption of wound exudate
5 = Mimics skin

For each of the five factors listed, a rank of one is the least desirable rank and five is the most desirable rank. These ranks have been tailored for evaluation of Biobrane® and porcine.

TABLE 2

E-Z Derm™ AND Biobrane®: WOUND DRESSING INDEX VALUES

Factors	Sites	E-Z Derm™	Biobrane®
Adherence	14	3.9	3.5
Conformation	14	3.7	3.9
Granulation Tissue	14	4.6*	2.8*
Pliability	14	3.6	4.2
Suppuration	14	4.5*	3.2*

*P < 0.005

RESULTS

The mean scores in each of the five categories assessed in the 14 paired sites are presented in Table 3. Statistically, significant differences were noted in two categories when comparing E-Z Derm™ and Biobrane®. Granulation tissue formation beneath the dressing at the time of definitive autografting was rated better on the E-Z Derm™ sites ($P < 0.05$). Nonhypertrophic, beefy red granulation tissue was felt to represent an optimal site for autograft take. Prior experience has demonstrated that removal of adherent dressing on the "best" sites was accompanied by brisk bleeding and some resistance to the removal of the test dressing. Graft "take" on each of these sites which progressed to autografting was evaluated, resulting in 93 and 80 percent for E-Z Derm™ and Biobrane®, respectively ($P < 0.05$). Episodes of suppuration beneath the test dressings were also significantly less frequent with E-Z Derm™ ($P < 0.05$), although both performed relatively well up to 14 days. Prior to 14 days postapplication, both materials were noted to have fluid collections on the two sites, necessitating local debridement. Additionally, fluid collections with frank purulence on three separate Biobrane® study sites were noted on postapplication days 15, 17, and 18, necessitating total Biobrane® removal from two sites and partial removal in the third. All fluid collections were cultured and correlated with the clinical course. In one patient with septicemia and pneumonia, Pseudomonas aeruginosa was cultured from beneath both dressing sites after removal for progressive suppuration. Positive cultures necessitated wet-to-dry treatment of the wound with

TABLE 3

PHYSICIAN PREFERENCE FOR FUTURE USE

	E-Z Derm™	Biobrane®
Definatively	6	3
Probably	1	3
Maybe	3	4
No	1	1

five-percent sulfamylon-soaked dressings twice daily until such time as biologic dressing could again be applied.

Both materials were highly rated in handling characteristics (conformation and pliability), receiving equivalent scores. Likewise, initial adherence was comparable. In these categories, however, considerable site specificity was noted. E-Z Derm™ performed better on flat anterior surfaces while Biobrane® performed better on dependent surfaces, particularly when applied circumferentially. E-Z Derm™ sites sustained fewer episodes of fluid accumulation beneath the dressing than Biobrane® and thus was rated higher. Dressings were permitted to remain in place until autografting, patient death, or suppuration. E-Z Derm™ remained adherent an average of 18.5 days (range = 8 to 46) and Biobrane® 13 days (range = 7 to 21). Suppuration beneath the dressing constituted the major reason for early removal of Biobrane®. The graftable fibrovascular bed was better achieved with E-Z Derm™ than Biobrane®, resulting in 93 and 80 percent graft "take," respectively. When primary physician preference was recorded at completion of each study patient, there was no significant difference noted between the dressings. No local or systemic effects were noted during the course of the study.

DISCUSSION

In recent years, biomedical technology has afforded the clinician caring for the thermally injured patient with a number of biologic dressings and skin substitutes for use when

wound coverage is problematic (4,9-10). This group includes patients where adequate donor sites or general condition does not permit definitive autografting following excision of nonviable tissue.

The necessary mechanical and physical properties of skin substitutes and biosynthetic materials available for topical wound closure have been outlined. Roberts et al (8) has previously defined characteristics important to temporary burn wound dressings and has outlined a wound dressing index for use in comparing various materials. Biologic dressing should control water evaporation and protein loss from the wound, support production of a graftable fibrovascular tissue bed, facilitate infection control at the wound interface, remain adherent, and conform to the wound surface until definitive wound autografting can be effected. Optimally, these materials should be easily stored, applied, and maintained. Autograft has been traditionally used for this purpose, but availability and cost can greatly limit its utility (11). Xenograft has thus subsequently enjoyed wide use and is generally more available than allograft, although this material, too, is not without certain limitations (12). Storage requirements, the need for frequent replacement, and the less than optimal handling characteristic of lyophilized and frozen preparations have inspired the search for alternative materials. Numerous biologic materials, synthetic polymers, and combinations of these have been marketed with fervor in recent years. A great deal of interest has been generated comparing the properties of the many new dressings now available. The recent advances in the field, however, militate for a standardized set of criteria for use in comparing various biologic dressings and skin substitutes. Only then can consistency be achieved in evaluations within and between burn centers. Data controlled with regard to each dressing can thus be analyzed more effectively to define important properties of each material and optimize their use.

In the current study, E-Z Derm™ proved superior to Biobrane® in two categories, i.e., the promotion of a graftable fibrovascular bed and the ability to prevent suppuration

---

[9]Robson MC and Krizek TJ: The effect of human amniotic membranes on the bacterial population of infected rat burns. Ann Surg 177:144-149, 1973.

[10]Lawrence JC: What materials for dressings? Injury 13:500-512, 1980.

[11]Namba K, Koga Y, Mukae N, et al: Clinical effects of allograft. JBCR 5:38-43, 1984.

[12]Wang H-J, Yeap CL, and Heimbach DM: Allograft vs. xenograft in preparation of wound for autograft. JBCR 5:116-118, 1984.

beneath the dressing, although both prove overall to be efficacious. Using a standardized wound dressing index, current and new biologic dressings and skin substitutes can be systematically evaluated in a prospective fashion with comparisons of desirable properties and a uniform grading system to facilitate analysis of results.

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH COMPLETION REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: The Influence Upon Electrolyte and Water Balance of Tap Water Showers in Burned Patients

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 22 August 1986

INVESTIGATORS

Marisa R. Herson, MD
Bryan S. Jordan, RN, Captain, AN
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: The Influence Upon Electrolyte and Water Balance of Tap Water Showers in Burned Patients

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 22 Aug 86

INVESTIGATORS: Marsia R. Herson, MD
Bryan S. Jordan, RN, Captain, AN
Arthur D. Mason, Jr., MD
Albert T. McManus, PhD
Basil A. Pruitt, Jr., MD, Colonel, MC

Hydrotherapy is now considered one of the traditional options in the treatment of burn wounds. Together with topical antimicrobial agents, hydrotherapy constitutes an integral part of wound management. Hydrotherapy has two disadvantages. The loss of barrier function in burned integument makes diffusional sodium and potassium depletion possible and the submergence of a patient in bath water with high concentrations of bacteria, permitting cross-contamination of unaffected areas of the burn are untoward effects of this therapy. These considerations have given rise to showering burn patients rather than submergining them in water. Though there are no documented untoward effects of showering patients, this study examines the influence of showering on serum electrolytes and water balance in burn patients.

THE INFLUENCE UPON ELECTROLYTE AND WATER BALANCE OF TAP WATER SHOWERS IN BURNED PATIENTS

INTRODUCTION

Electrolyte and fluid imbalance are known complications of burns. Fluids and salts are mainly lost due to capillary leakage into the so-called "third space," especially in the acute phase of injury. Also, sodium loss through the wound is increased (1). Cohen (2) studied the exudative water loss from eschar, granulation tissue, and intact skin. All three areas showed increased evaporative losses until covered by skin (either homografts, autografts, or spontaneous healing). Moser et al (3) showed not only loss from the wounds to the environment, but that denuded areas absorb electrolytes and water from surrounding media. Such electrolyte exchange is related to the difference between the concentration of the salts in the patient's plasma and in the bathing media. Wolfe et al (4) described an exchange of potassium and urea between granulation tissue and bathing water when an electrolyte gradient was present. He commented on the importance of considering this phenomena when bathing a burn patient in hypertonic or hypotonic solutions. Sodium loss through granulation tissue when using 0.5-percent silver nitrate solutions as topical treatment is a classical example of the permeability of denuded tissues (5).

Hydrotherapy is now considered a traditional method for the treatment of burn wounds. Bathing may be of benefit in the maceration and separation of eschar for local debridement and presents an ideal opportunity for physiotherapy. This method of wound care has two distinct disadvantages, sodium and

---

[1]Batchelor ADR, Sutherland AB, and Clover C: Sodium balance studies following thermal injury. Br J Plast Surg 18:130-145, 1965.

[2]Cohen S: Investigation and fractional assessment of evaporative water loss through normal skin and burn eschar using a microhydrometer. Plast Reconstr Surg 37:475-486, 1966.

[3]Moser MH, Robinson DW, and Schloerb PR: Transfer of water and electrolytes across granulation tissue in patients following burns. Surg Gyn Obstet 118:984-988, 1964.

[4]Wolfe JJ, Noland JL, and Stratford B: Passage of solutes across experimental burns. J Trauma 5:535-539, 1965.

[5]Moyer CA, Brentano L, Gravens DL, et al: Treatment of large human burns with 0.5% silver nitrate solution. Arch Surg 90:812-867, 1965.

potassium depletion as suggested by Gotshall (6) and exposure to high concentrations of bacteria in the bathing media as described by Moyer et al (5). To obviate untoward effects, showers have been used to effect wound cleansing. In this study, pre- and postshower serum and urine electrolytes, weight, length of shower time, and other pertinent data have been assessed.

MATERIALS AND METHODS

Three adult patients with at least 30-percent total body surface area burns and normal renal function were entered into this study. One hour prior to the showering procedure, serum and urine were collected to determine the concentrations of sodium, potassium, chloride, and calcium. In addition, total urine output and fluids and medications administered were recorded. Just prior to showering, pulse, temperature, respiration, and blood pressure were obtained in addition to shower room temperature and humidity.

The denuded areas of each patient were recorded, including eschar-covered areas, granulating areas, and donor sites if less than seven days old. Body weight (with as few dressings as possible) was recorded and a specimen of the tap water was obtained for analysis for dissolved electrolytes. The duration of the shower was also recorded. After the shower, the patient was reweighed. One hour after the shower, serum and urine were again collected for determination of sodium, potassium, chloride, and calcium concentrations and urine output, fluids, and any medications administered were recorded.

RESULTS

The change in electrolyte and free water concentrations in serum and urine were assessed in burned patients undergoing hydrotherapy in the shower. Showering patients, although routine during the convalescent phase of burn injury, is infrequent during the acute phases of treatment. The trend toward excision and grafting of wounds as soon as the patient's condition allows leads to the fact that extensively burned patients (> 50-percent total body surface area burns) are not generally showered before undergoing several grafting procedures. Also, many patients who are showered do not require indwelling urinary catheters. Thus, the population available for this study was limited to only three patients; seven sets of data were collected.

---

[6]Gotshall RA: Sodium depletion related to hydrotherapy for burn injury. JAMA 203:182-184, 1968.

Serum Specimens. There was a trend toward a decrease in serum sodium, potassium, chloride, and calcium. Also, a moderate increase in glucose levels was noted. No significant changes in urea were noted. Blood osmolality decreased slightly.

Urine Specimens. A notable increase in urinary sodium was noted after the showering procedure. Urine volumes appeared to be decreased postshowering.

Hemodynamics. Blood pressures were generally increased after the procedure, mostly in the diastolic phase.

DISCUSSION

In a previous study, 80 13 milliliters of water per square meter were shown to be absorbed by granulation tissue (1). At the same time, sodium salts could be lost to the bathing medium. This may explain the dilution and/or depletion effect on serum sodium with a decrease in sodium levels and blood osmolality. As a consequence of the hyponatremia, hormonal pathways for renal sodium reabsorption would be activated, resulting in lowered urinary sodium as noted.

However, kaliuresis was not observed at significant levels nor was there any dilution effect, as measured by the absence of change in body weight. Sodium and water retention and elevation in serum glucose and blood pressure might be physiological responses to stress associated with the showering procedure.

We feel this study should be extended by studying patients without open wounds who are bedridden or on bedrest and who have indwelling catheters. Isotonic solutions may offer advantages as cleansing and rinsing solutions.

RESEARCH TERMINATION REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: Reduction of Blood Loss During Tangential Excision of Burn Wounds

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Stephen M. Pratt, MD
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: Reduction of Blood Loss During Tangential Excision of Burn Wounds

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: Stephen M. Pratt, MD
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Primary operative excision of the burn wound has, in many cases, replaced more conservative debridement after spontanteous separation of the eschar. A major complication of this technique, however, is the amount of blood loss incurred. Various topical agents have been proposed as ways of effecting hemostasis after excision prior to wound coverage.

Arginine vasopressin is a synthetic analogue of the posterior pituatary hormone which causes smooth muscle contraction. Particularly effective in causing smooth muscle contraction in arterioles, venules, and capillaries, it has the theoretical advantage of limiting hemorrhage by this constrictive effect.

Topical application of arginine vasopressin was compared to the standard therapy of topical thrombin after excision in four patients. Both agents performed equally well. Further study was aborted because of the study's effect on operative technique and time, changes in the preferred method of thrombin application, and concurrent reports dealing with this subject.

REDUCTION OF BLOOD LOSS DURING TANGENTIAL EXCISION OF BURN WOUNDS

INTRODUCTION

Since the description by Janzekovic (1) of tangential excision of nonviable tissue resulting from burn injury, this technique has been employed in both wound excision and debridement of retained nonviable tissue. Theoretically, it is appealing to consider debridement of such nonviable tissue followed by prompt closure of the excised wound with viable cutaneous autograft as potentially reducing hospital stay, morbidity, and mortality. The difficulties inherent in tangential excision include massive blood loss, absence of a reliable method to determine whether all nonviable tissue has been excised, and predictability of viable autograft skin survival following transfer to such excised wounds. Loss of autografted skin, wound infection and invasion, and acute blood loss are significant risks of this technique.

In an attempt to reduce the intraoperative blood attending an adequate level of excision, we have studied the use of topical application of arginine vasopressin mixed in saline following tangential excision. Arginine vasopressin applied to the wound after excision was compared to the current method of topical thrombin application in paired sites.

MATERIALS AND METHODS

Patients eligible for study were those admitted to the Institute with full-thickness burn wounds requiring tangential excision. Patients who had a known hypersensitivity to vasopressin, a history of coronary artery disease, hypertension, or epilepsy were excluded from study entry.

Sites were chosen which were comparable with respect to anatomic location, depth of injury, and size. Two 10 X 20 centimeter areas of burn eschar were tangentially excised to the level of viable tissue as determined by the presence of fine punctate bleeding. Following excision, the areas were covered for five minutes with five telfa pads soaked with either 50 milliliters of normal saline containing six units of vasopressin or 50 milliliters of thrombin solution.

The telfa pads were subsequently removed, placed in individual containers, and transported to the laboratory for determination of hemoglobin content. After 1:10 dilution to

---

[1]Janzekovic Z: The treatment of burns: excision of burns. Burns 4:61-66, 1977.

facilitate analysis, hemoglobin content of the telfa pads was assessed spectrophotometrically. Assays were performed in triplicate, the mean values being used for comparison.

RESULTS

Four patients were entered into the study. Results of hemoglobin determinations are shown in Table 1. No significant differences of hemoglobin content of the applied telfa pads were noted, nor did clinical observation indicate any difference in bleeding between the sites.

DISCUSSION

During the course of this study, it quickly became apparent that no differences existed between topical vasopressin application and thrombin application with regard to the control of hemostasis. Additionally, it was felt that the technique used to create excised wounds of equal size and the need to measure blood loss from each site placed undue constraints on the operative procedures and unacceptably prolonged operative time.

During the study period, the method of applying thrombin was also revised. Aerosolized thrombin sprayed directly onto the wound was employed with results superior to topical application of the substance. This method rapidly achieved favor amoung the operating surgeons at the Institute, and thus, a return to topical application using telfa pads was viewed as undesirable.

Additionally, the subject of topical therapy to the excised burn wound to hasten hemostasis and reduce blood loss has received much attention recently. Reports using models similar to the one employed here have repeatedly documented the efficacy of topical thrombin, epinephrine, and vasopressin. Two investigative teams presented their experience with vasopressin and epinephrine at the Eighteenth Annual Meeting of the American Burn Association earlier this year, espousing the benefits of each (2-3). Thus, many of these agents have proved to be useful for the purpose outlined, making availability and physician preference the ultimate determinants of use.

---

[2]Brezel BS, McGeever KE, and Stein JM: Topical epinephrine solution vs. thrombin solution for hemostasis on split thickness skin donor sites. Proceedings of the American Burn Association (Abstract 77), 1986.

[3]Achauer BM, Hernandez J, and Parker A: Minimizing blood loss in primary excision with intraoperative vasopressin. Proceedings of the American Burn Association (Abstract 77), 1986.

TABLE 1. Hemoglobin Values

Patient Number	Thrombin Solution Hemoglobin (mg)	Vasopressin Solution Hemoglobin (mg)
1	26.8	25.8
	24.3	24.6
	24.8	25.0
PATIENT'S MEAN VALUE =	25.3	25.1
2	14.1	14.8
	14.6	15.9
	13.8	16.1
PATIENT'S MEAN VALUE =	14.2	15.6
3	14.9	12.2
	15.6	12.9
	16.8	13.4
PATIENT'S MEAN VALUE =	15.8	12.8
4	38.4	40.4
	34.7	38.6
	34.9	36.1
PATIENT'S MEAN VALUE =	36.0	38.4
OVERALL MEAN VALUE =	22.8	23.0*

*$P > 0.1$

PRESENTATIONS/PUBLICATIONS

None.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: A Study to Evaluate the Effectiveness and Safety of Artificial Skin in the Treatment of Third Degree Flame or Scald Injuries

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Stephen M. Pratt, MD
Bryan S. Jordan, RN, Captain, AN
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: A Study to Evaluate the Effectiveness and Safety of Artificial Skin in the Treatment of Third Degree Flame or Scald Injuries

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: Stephen M. Pratt, MD
Bryan S. Jordan, RN, Captain, AN
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Evaluation of the safety and efficacy of Artificial Skin in the treatment of third degree flame and scald burns is being conducted at this Institute as part of a multicenter study. Artificial Skin, a bilaminate membrane which consists of distinct dermal and epidermal analogues, is being compared to standard burn wound covers, i.e., autograft, allograft, xenograft, and synthetic materials, as a wound bed cover following excision of full-thickness burn wounds.

Five patients have been entered into the study during this period. The average total body surface area burn was 47 percent (range = 24 to 70). Artificial Skin was used on sites averaging nine percent of the total body surface area (range = 6 to 10.5). Control sites in all cases to date have been autografted. Mean healing time was 37 days (standard deviation 10.6) for Artificial Skin sites after definitive epidermal autografting and 36 days (standard deviation 24.75) for control autograft sites.

Complications with the use of Artificial Skin include total loss of the material secondary to infection (one patient), premature separation of a portion of the Silastic® component (three patients), shallow wrinkling in the initial postoperative period (one patient), and hematoma beneath the dressing requiring replacement (one patient). No deaths or septic complications attributable to the material have been observed. Further evaluation of at least 15 more patients will

be necessary for the efficacy and utility of Artificial Skin to be properly assessed.

A STUDY TO EVALUATE THE EFFECTIVENESS AND SAFETY OF ARTIFICIAL SKIN IN THE TREATMENT OF THIRD DEGREE FLAME OR SCALD INJURIES

INTRODUCTION

Tangential excision and autografting are standard procedures utilized in modern burn care. However, in extensive burn injury, limited autograft availability is a problem. Repeated autograft harvesting and the necessity to apply widely meshed autograft to achieve coverage provide less than optimal long-term coverage. The promotion of a "scar epithelium" with a tendency for contracture, breakdown, and poor cosmesis is the end result. To date, no acceptable permanent replacement for autograft has been developed, although biologic dressings and skin substitutes are now extensively used to meet the immediate needs of wound coverage when autograft is not available (1-7). Artificial Skin (Integra®, Marion Laboratories, Kansas City, MS), unlike any prior material, was designed to achieve the advantages of immediate coverage and to provide a superior long-term skin analogue (8).

Artificial Skin is a bilaminate material consisting of distinct dermal and epidermal analogues. Composed of a polymer of chondroitin-6-sulfate and collagen, the dermal portion provides a template for the ingrowth of capillaries and fibroblasts, forming a "neodermis," after which definitive

---

[1]Pruitt BA Jr and Levine NS: Characteristics and uses of biologic dressings and skin substitutes. Arch Surg 119:312-322, 1984.

[2]Hermans MHE and Hermans RP: Preliminary report on the use of a new hydrocolloid dressing in the treatment of burns. Burns 11:125-129, 1984.

[3]Lawrence JC: What materials for dressings? Injury 13:500-512, 1981.

[4]Levine NS, Lindberg RA, Salisbury RE, et al: Comparison of coarse mesh gauze with biologic dressings on granulating wounds. Am J Surg 131:727-729, 1976.

[5]Nathan P, Robb EC, Dressler D, et al: A silicone-nylon laminated dressing (IP-758) for closure of excised or debrided burn wounds. Burns 8:328-332, 1981.

[6]Sagi A, Walter P, Walter MH, et al: Dermodress: a new, temporary skin substitute for extensive deep burn coverage. Plast Reconstr Surg 75:223-226, 1985.

[7]Barlett RH: Skin substitutes. J Trauma 21:731-732, 1981.

[8]Burke JF, Yannas IV, Quinby WC, et al: Successful use of a physiologically acceptable artificial skin in the treatment of extensive burn injury. Ann Surg 194:413-428, 1981.

epidermal grafting can be effected (9-11). The outer epidermal portion is designed to meet the immediate short-term need for a temporary wound cover, i.e., control of water and protein loss, reduction of heat loss, provision of a barrier to bacterial invasion, and pain control. The outer 0.1-millimeter thick Silastic® sheet of Artificial Skin is designed to be removed after formation of the neodermis, at a time when the patient's condition permits epidermal autografting. Unlike autografting in the traditional sense, which has a significant dermal element, the recipient neodermis requires only a thin epidermal graft (0.004 inch). The end result is coverage with a better dermal base than is normally achieved when donor sites are limited. The donor site has also been reported to heal more rapidly, allowing for more frequent epidermal harvests (9-11).

Artificial Skin is being used in a prospective trial on paired full-thickness burn wounds following excision. The ability of Artificial Skin to effect immediate wound coverage, the healing time of Artificial Skin sites, the quality of healed sites, adverse reactions, and effects on patient management are assessed. Subjective evaluations are also completed by each study patient.

MATERIALS AND METHODS

Patient Criteria. Patients eligible for entry into this study are those hospitalized with extensive thermal injuries which are life-threatening and/or cover at least 10 percent of the total body surface area and, in the opinion of the investigator, will not heal within three weeks and are amenable to excisional therapy. All patients are hospitalized within 24 hours of the burn and undergo excisional therapy of their wounds. The wounds utilized for evaluation are similar in size and site. Excision of the wound is initiated within seven days of injury and is completed within 21 days of injury.

Baseline Evaluations. All patients undergo prestudy screening to determine eligibility for study entry. If the patient is qualified for the study, a history is taken and a physical examination performed. The size of the burn is estimated and recorded. Blood and urine cultures are taken when clinically indicated. Laboratory studies include

---

[9]Yannas IV and Burke JF: Design of an artificial skin. I. Basic design principles. J Biomed Matr Res 14:65-81, 1980.

[10]Yannas IV, Burke JF, Gordon PL, et al: Design of an artificial skin. II. Control of chemical composition. J Biomed Matr Res 14:107-131, 1980.

[11]Dagalakis N, Flink J, Stasikelis P, et al: Design of an artificial skin. Part III. Control of pore structure. J Biomed Matr Res 14:511-528, 1980.

hematological studies with a complete blood count with differential, SMAC (glucose, BUN,creatinine, sodium, potassium, chloride, bicarbonate, uric acid, calcium, phosphate, SGOT, total protein, albumin, and alkaline phosphatase), total and direct bilirubin, and complete urinalysis.

Wound Management. Burn wounds are excised to the level of viable tissue as judged by color, texture, and the presence of punctate bleeding. Both tangential and fascial excision are employed as appropriate. Tourniquets, topical thrombin, and warm laparotomy sponges are used to effect hemostasis. Using a computer generated randomization scheme, comparative sites are designated to receive either Artificial Skin or control coverage. Autograft is meshed either 1.5:1 or 3.0:1 and expanded as appropriate. Prior to coverage, each site is biopsied for microbiologic assessment. Autograft and Artificial Skin are stapled into place using small surgical clips prior to coverage with an occlusive dressing treated with five-percent aqueous mafenide acetate. Immobilization as appropriate is also used.

Autograft site dressings are removed on either the third or fourth postoperative day. Artificial Skin dressings are removed on the third postoperative day and the site is subsequently left exposed. Fluid collections beneath the dermal element or the Silastic® are aspirated and cultured. Nonadherent areas are sharply debrided. At approximately 14 days after application, epidermal autografting is performed. Under general anesthesia, epidermal grafts are taken with the Padgett dermatome set at 0.004 inch. These are meshed 1.5:1 and applied to the neodermis after Silastic® removal. Epidermal grafts are dressed with a single layer of fine mesh gauze followed by an occlusive layer of coarse-mesh gauze treated with five-percent aqueous mafenide acetate. Because of the extremely friable nature of these thin grafts, dressings are allowed to remain in place until the fifth postoperative day, at which time the course mesh layer is removed. Clean fine mesh gauze is permitted to remain in place until the seventh to tenth postoperative day. Occlusive dressings are employed at this stage for approximately two more weeks.

Effectiveness Evaluation. Artificial Skin is compared to conventional covers. The primary end point for evaluating the efficacy of Artificial Skin is the time from placement of the definitive epidermal autograft to final closure. Additionally, the "take" of Artificial Skin to the wound bed, the adherence of the Silastic® "epidermis" to the "dermal" element, and the "take" of the final epidermal graft to the neodermis are evaluated for Artificial Skin. The "take" of the other dressing to the wound bed and the "take" of the definitive autograft are evaluated. The method of removal of nonautograft dressing and the difficulties encountered in their removal are

recorded. Another parameter assessed is infection at the sites. Following excision, the wounds are cultured, following removal of the Silastic®, the dermis is cultured, and following removal of the comparative cover at the time of autograft, the wound is cultured. At any time when clinical evidence of sepsis or infection is apparent, a culture of both the Artificial Skin and the comparative site are obtained from the area that is clinically determined most likely to be infected.

Rates of wound healing are compared between the two materials used. Both wounds are rated after final healing as to percent of autoepidermal or autograft "take" and serviceability of the cover. Comparisons include the cosmetic appearance of the wound sites. Photographic documentation of all sites is obtained. Subjective evaluations by the participating patient and the investigator are made at one and two-month periods after initial placement of the material.

Safety Evaluation. Patient safety is evaluated by obtaining vital signs at a minimum of every six hours until the patient is felt to be clinically normal. Intake and output are continuously monitored at a minimum of every 24 hours. SMAC profiles, routine hematologies, and urinalysis are obtained just prior to the first application of Artificial Skin, weekly thereafter until all comparative and the noncomparative study sites are grafted and healed, and on the last day of hospitalization. Each patient has blood samples drawn for determination of antibody formation to Artificial Skin, bovine collagen types I, II, and III, and human collagen types I, II, and III. Tissue samples are taken by biopsy from the sites at which Artificial Skin was applied for histologic evaluation of the neodermis at defined intervals during the initial two months after application. Adverse reactions, including both clinical events and laboratory values attributable to the use of Artificial Skin, are noted.

Analysis of Results. The various assessment parameters that will be considered in this study include time to final wound cover, percent "take" of autograft, percent "take" of Artificial Skin, level of bacterial invasion at each site, relationship of septicemia to site invasion, incidence of adverse reactions, immunological changes (tissue and/or blood), final cosmetic outcome, and patient's subjective assessment. Application of the Fisher Sign Test, in most cases, will be used to test against the null hypothesis that no difference exists between Artificial Skin and control materials. The student's t-test will be used where applicable to healing times.

RESULTS

Five patients (four male, one female) have been entered into the study. Final evaluations are complete on the initial four patients. Mean patient age was 30 years (range = 21 to 38). Average total body surface area burn size was 47 percent (range = 24 to 70) with an average full-thickness burn size of 31 percent (range = 18 to 45). All patients received initial placement of the Artificial Skin and control cover within six days postburn. Control sites in the initial five patients have been 0.015 inch autograft meshed 1.5:1 (four patients) and 3:1 (one patient).

Figure 1 shows the excision to viable tissue, while Figure 2 shows the application of Artificial Skin and control dressings to full-thickness burn wounds. Figure 3 depicts the postoperative result in one patient who underwent autograft placement to the right chest (control site) and Artificial Skin application to the left chest. Following initial excision and Artificial Skin placement, definitive autografting of the neodermis was conducted as determined by an obligatory 10 to 14-day period for graftable neodermis formation, availability of donor sites, remaining wound status, and overall patient condition. Neodermis following Silastic® removal has the appearance of a smooth, uniform fibrovascular bed (Figure 4) to which harvested epidermal autografts (0.004 inch thick) are applied (Figures 5 and 6). Although vigilance is necessary so as not to damage these extremely delicate grafts, technique is otherwise similar to traditional autografting.

Table 1 depicts the period of Artificial Skin application prior to epidermal grafting, the time to effect a "healed" wound after grafting, and the number of procedures necessary before total closure of each study site. The end point of final closure denotes 100-percent epithelial coverage exclusive of subsequent breakdown secondary to trauma, blistering, etc., which in some cases resulted in prolonged hospitalization or the need for further operative intervention. The coverage achieved with epidermal autografting appears to be much the same as traditional autografting in the early period after wound closure (Figure 7). Artificial Skin and autograft "take" to the excised bed after the initial procedure and epidermal autograft "take" to the neodermis after Silastic® removal are shown in Table 2.

Complications. Fluid collection beneath the Silastic® layer and separation of the Silastic® from the dermal analogue have been major complications to date (Table 3). Total Silastic® separation before epidermal autografting occurred in patients 1 and 2, necessitating coverage of the neodermis with biologic dressing and the utilization of five-percent mafenide acetate soaks. Silastic® separation in patients 3 and 4 prior

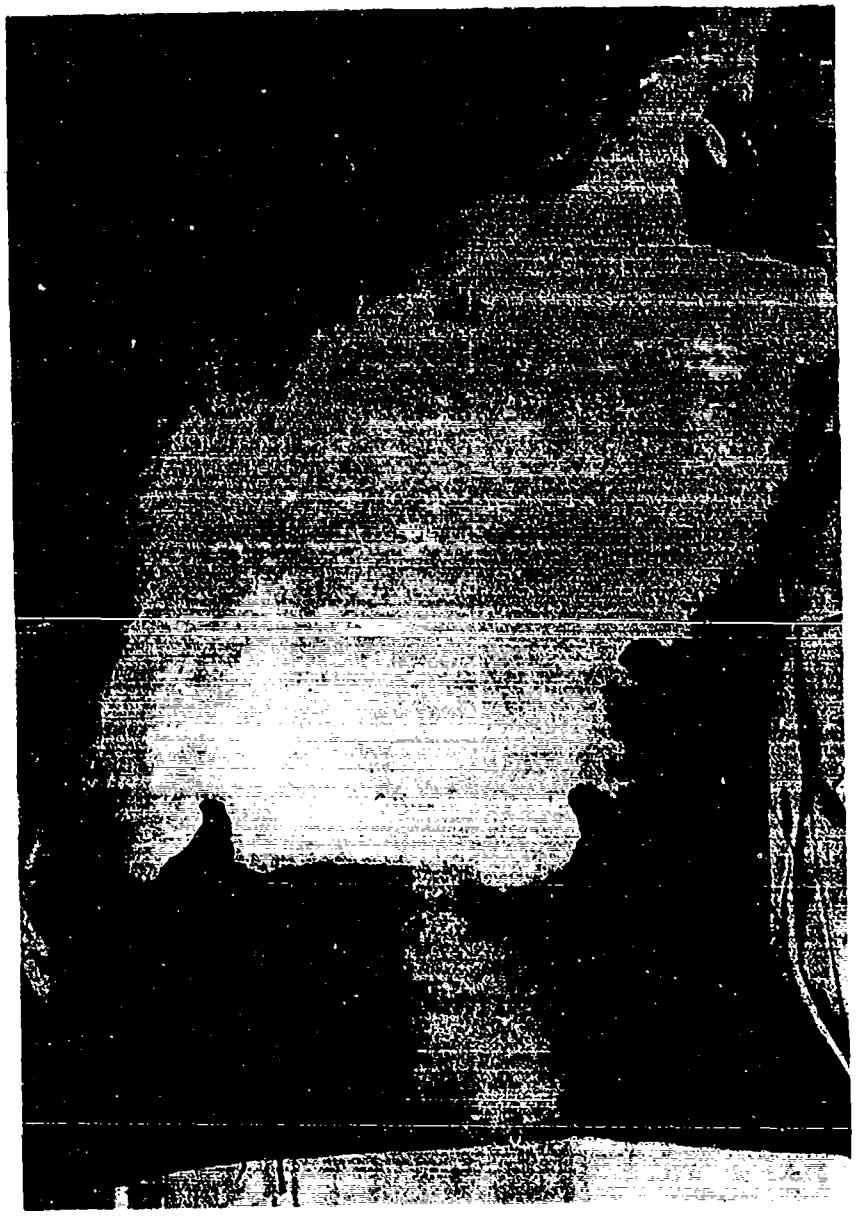


FIGURE 1. Patient depicted in Figure 1 after tangential excision to level of viable tissue.

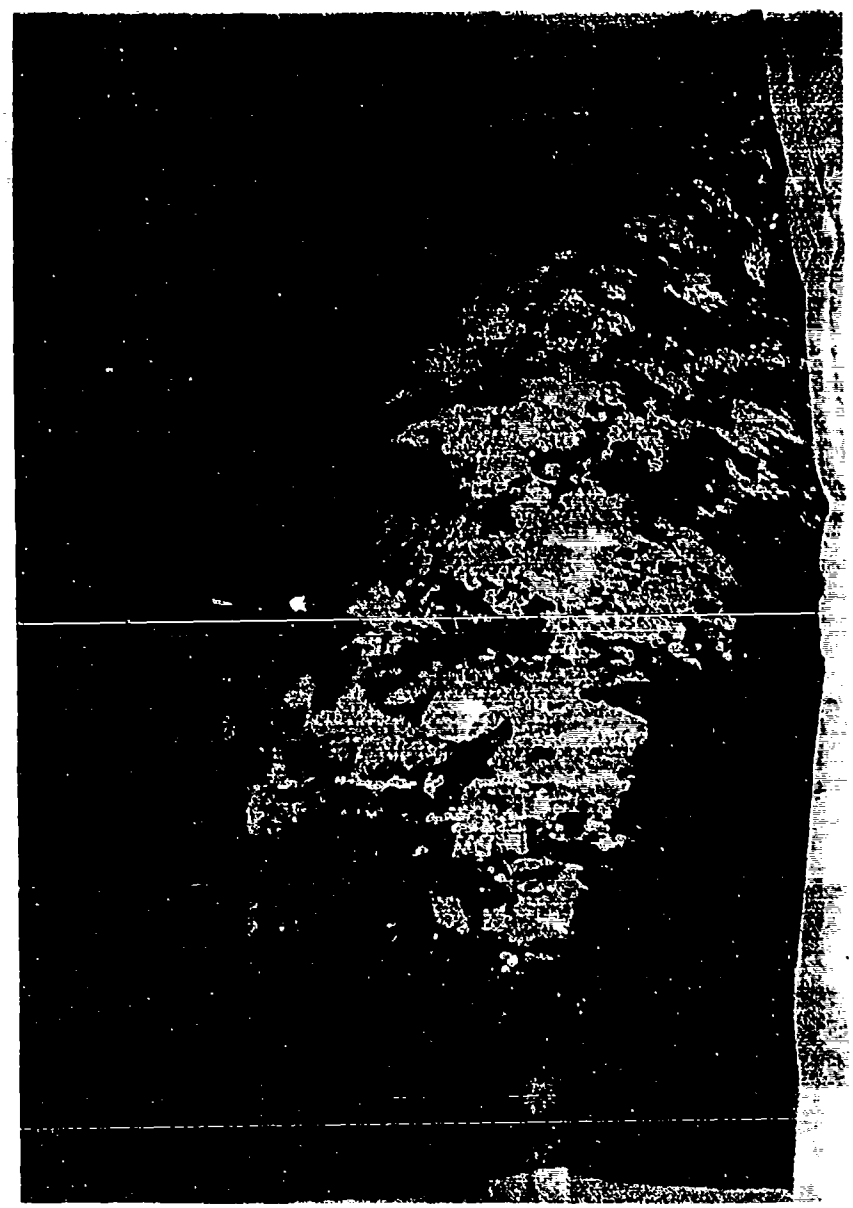


FIGURE 2. Full-thickness burn wound six days after house fire.

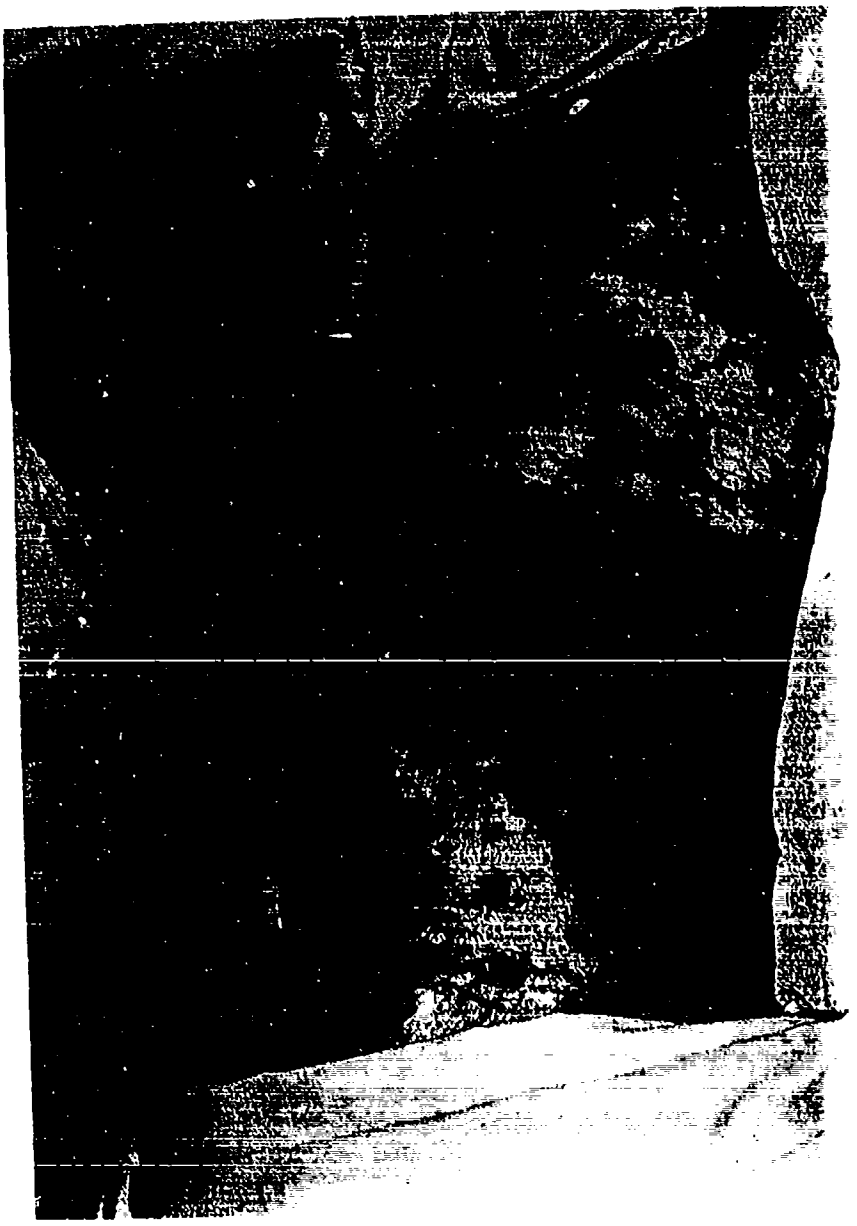


FIGURE 3. In accordance to the randomization scheme, Artificial Skin has been applied to the left chest and control material (in this case autograft) has been placed on the left chest.

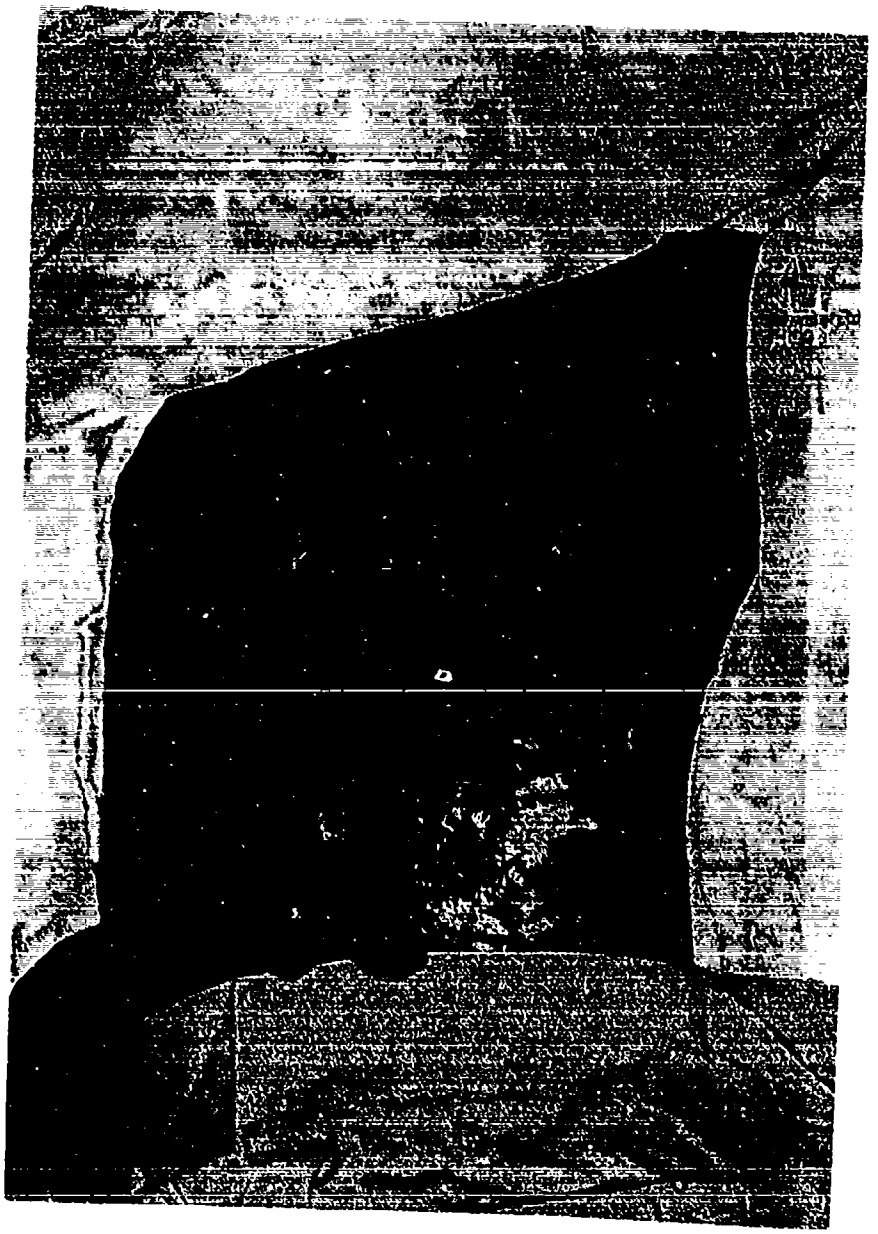


FIGURE 4. Neodermis after Silastic^{R} removal 19 days after Artificial Skin placement. The bed has a characteristic appearance not unlike granulation tissue and appears to be the ready recipient of a graft.

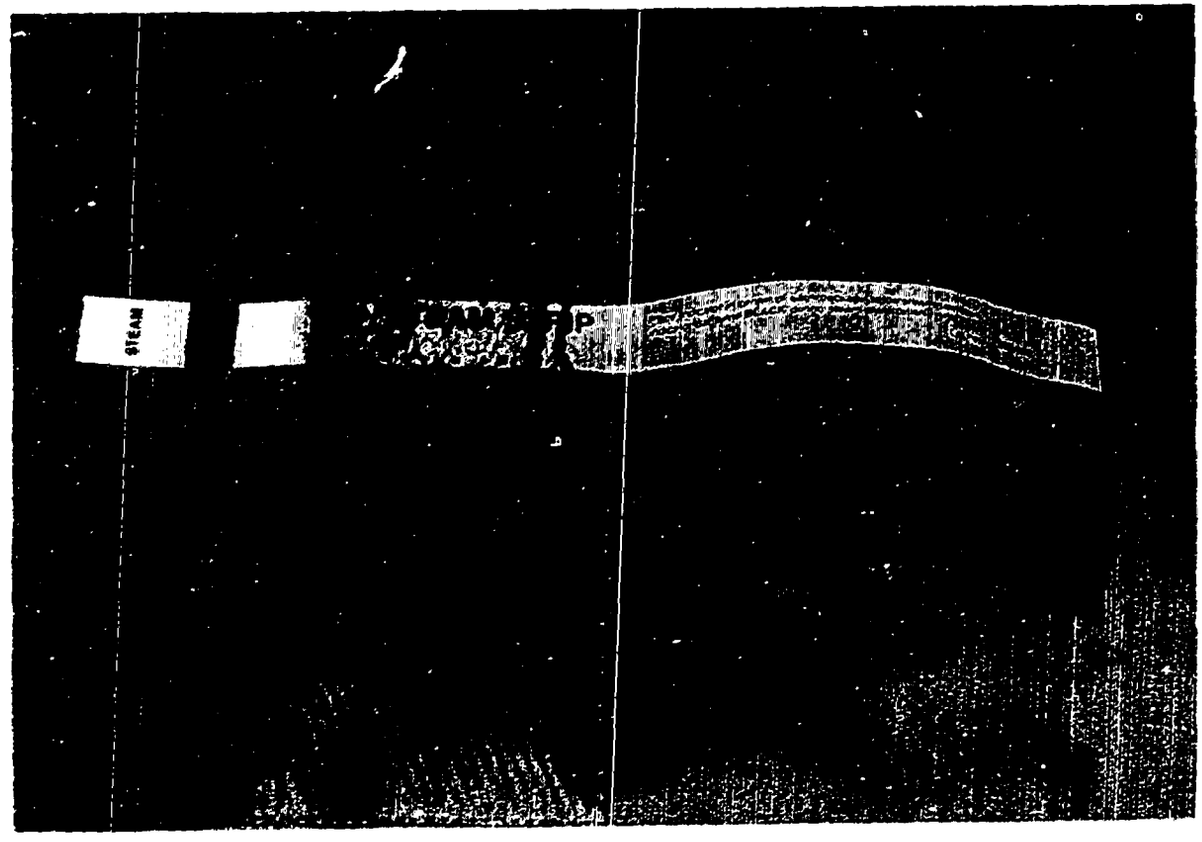


FIGURE 5. Epidermal autograft harvested with the Padgett dermatome set at 0.004 inch. The composition of this thin graft is mostly epidermal with very little dermis present.

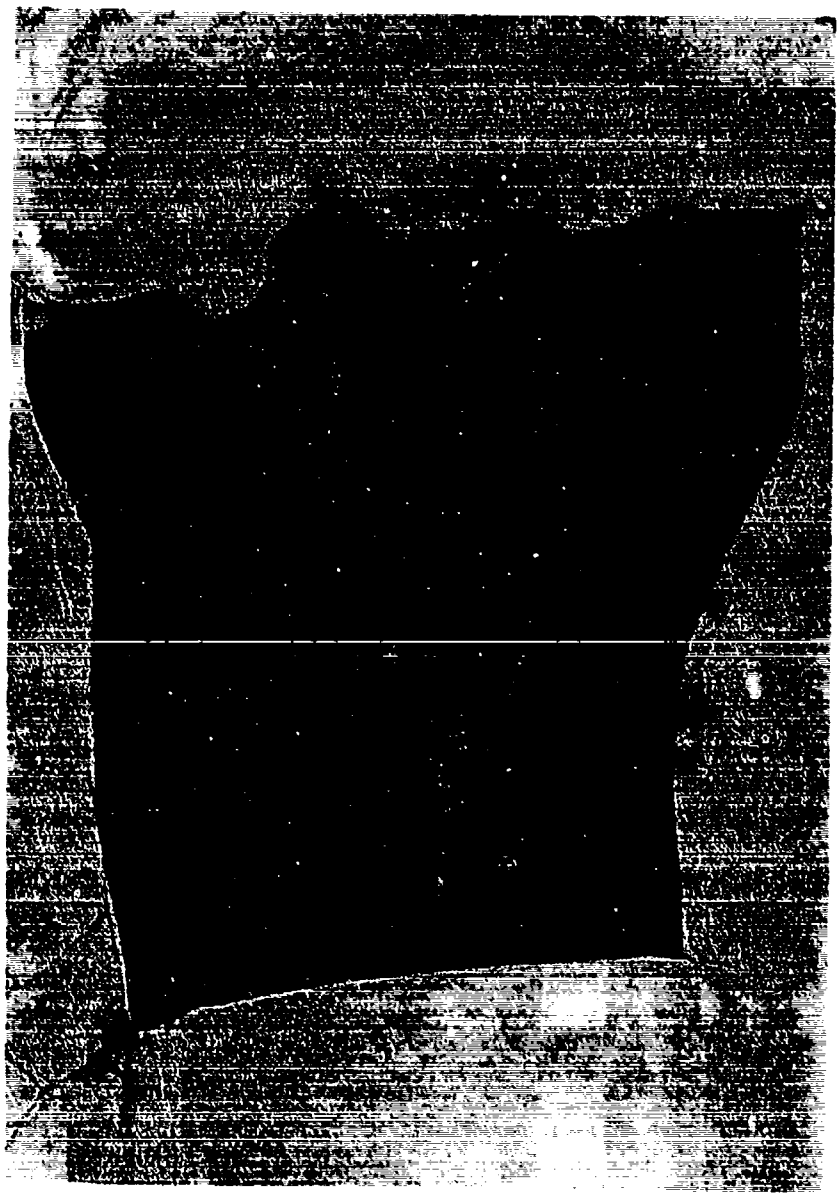


FIGURE 6. Epidermal autografts applied to the neodermis and stapled into place prior to dressing. Note the control site on the right chest that has already healed.

TABLE 1. Operative History

PATIENT NUMBER	ARTIFICIAL SKIN PLACEMENT TO EPIDERMAL AUTOGRAFTING (DAYS)	HEALING TIME (DAYS)		NUMBER OF OPERATIVE PROCEDURES	
		Artificial Skin Site*	Control Site	Artificial Skin Site**	Control Site
1	26	27	14	3	1
2	21	30	16	3	1
3	37	44	63	3	3
4	14	49	51	3	3

*Time from SilasticR removal to complete epidermal healing.
**Includes operation for initial Artificial Skin placement. 3:1 mesh/expanded.

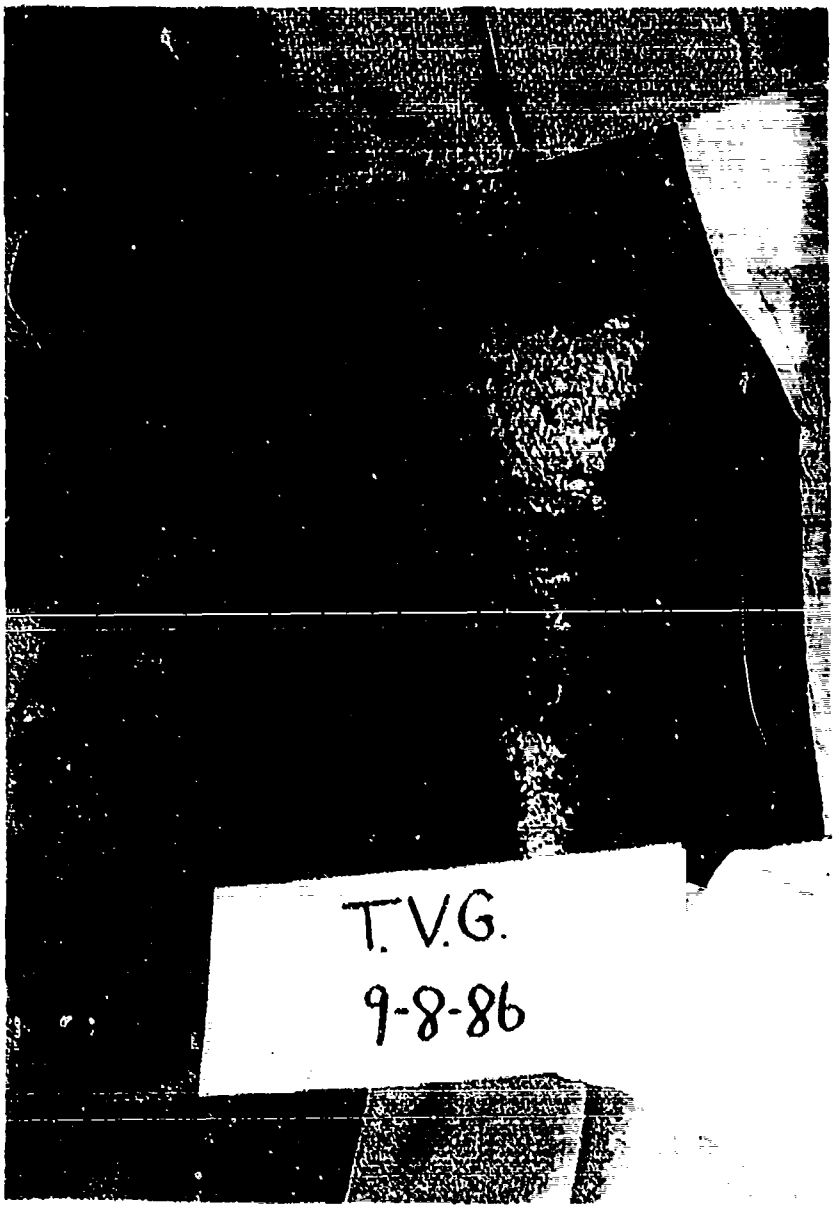


FIGURE 7. Control and Artificial Skin sites three weeks after final epidermal autografting. Differences in color are the result of grafts taken from previously harvested donor sites.

TABLE 2. Graft "Take" After Initial Procedure (Percent)

Patient Number	ARTIFICIAL SKIN: Artificial Skin to Graft Bed	ARTIFICIAL SKIN: Epidermal Autograft to Neodermis	CONTROL SITE
1	100	75	100
2	100	70	100
3	100	90	90
4	100	80	88
AVERAGE =	100	79	94.5

TABLE 3. Silastic^{R} Separation from Neodermis (Percent)

Patient Number	One Week After Application	Two Weeks After Application	At Time of Autografting
1	-	20	100 (26)
2	-	40	100 (21)
3	-	-	10 (37)
4	-	5	5 (14)

( ) = Postoperative Day

to epidermal autografting was 10 percent and five percent, respectively (Figure 1). Small fluid collections have usually been in areas of wrinkling and have been sterile in all cases. In patient 3, who had Artificial Skin placement on postburn day three, wrinkling was a prominent feature and attributed to a postresuscitation decrease in peripheral edema. "Take" of Artificial Skin to the excised bed was unaffected in this patient, yet wrinkling of the Silastic^{R} required frequent evacuation of fluid and sharp debridment of the wrinkled areas.

In patient 1, postoperative bleeding required evacuation of a hematoma from a previously dry graft bed on both control and Artificial Skin sites one day postoperatively. Reapplication of autograft and Artificial Skin resulted in 100-percent "take"

on both sites. No septic complications have resulted to date. Complications are summarized in Table 4.

Donor Sites. Donor site healing times were recorded in all cases for the purpose of comparing differences between the traditional thickness autografts (0.015 inch) and epidermal autograft (0.004 inch) used to graft the neodermis. In most cases, because control sites were autografted at the initial operative procedure placement, these sites were previously unharvested (eight sites) or had only one prior harvest (two sites). Epidermal autografts to the neodermis were initial harvest (one site), second harvests (six sites), third harvests (five sites), and fourth harvest (one case) of the same site. Healing times for each donor site thickness are depicted in Table 5. Donor site healing was constant regardless of the number of prior harvests. Without regard to prior harvests, thin autograft donor sites healed faster than thick autograft donor sites, 9.6 days and 13.6 days, respectively ($P < .001$).

Subjective Evaluation. No significant site preference was noted at the one-month or two-month physician evaluation. Two patients preferred control autograft sites to Artificial Skin sites, one patient preferred the Artificial Skin site, and one patient expressed no preference.

Immunology and Biopsy Results. Results are pending outside evaluation.

DISCUSSION

The relatively small size of the study population to date precludes reaching any definitive conclusions regarding to the overall safety and efficacy of Artificial Skin. Experience has, however, provided a data base for which further patient study can be accomplished during the next reporting period. Additional questions regarding a target patient population for Artificial Skin, optimum manners in which it is to be used to maximize its unique properties, and its overall impact on patient management and outcome will be answered. Only by further prospective analysis can these and other important questions be answered in a meaningful sense.

PRESENTATIONS/PUBLICTIONS

None.

TABLE 4. Complications with the Use of Artificial Skin

Patient Number	SilasticR Loss Prior to Autografting	Fluid Accumulation Beneath Silastic	Wrinkling of Artificial Skin	Hematoma
1	100%	3X	No	Yes
2	100%	2X	Yes	Yes
3	10%	8X	No	No
4	5%	1X	Yes	No

*P < .001

TABLE 5. Donor Site Comparisons

Prior Harvesting	Epidermal Autograft (0.004 Inch)	Standard Autograft (0.015 Inch)
0	1/8	8/13.5
1	6/9	2/14
2	5/10.4	-
3	1/11	-
TOTAL	13/9.6	10/13.6

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S62772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: 5% Aqueous Sulfamylon® Soaks Used in Topical Treatment of Burned Soldiers

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: 5% Aqueous Sulfamylon® Soaks Used in Topical Treatment of Burned Soldiers

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

During this reporting period, five-percent aqueous mafenide acetate-soaked (Sulfamylon®) dressings have continued to be an efficacious treatment modality in the care of the burn wound. One hundred and fifty-seven patients were treated with five-percent aqueous mafenide acetate-soaked dressings, employed either for final debridement of a wound or following application of meshed cutaneous autograft to prevent desiccation of tissue exposed in the interstices of such grafts. A 10.8-percent incidence of skin rash (atopy) was noted as the only adverse reaction. The clinical results achieved by the use of five-percent aqueous mafenide acetate solution strongly support its continued use.

BURN INJURY
TOPICAL THERAPY
VOLUNTEERS
5% MAFENIDE ACETATE SOLUTION

5% AQUEOUS SULFAMYLON[R] SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS

During this reporting period, the evaluation of five-percent mafenide acetate solution for topical treatment of the burn wound has continued at this Institute where it was used in 157 of 197 patients (79.7 percent). The five-percent mafenide acetate-soaked dressings are used as wet-to-dry dressings to debride nonviable tissue elements in preparation for split-thickness autograft procedures or as continuous wet dressings to protect freshly excised wounds that are not autografted. In addition, when meshed cutaneous autografts are applied, dressings are soaked with five-percent mafenide acetate solution to decrease the rate of bacterial growth and to prevent desiccation of tissue exposed in the interstices of such grafts.

Seventeen patients (10.8 percent) demonstrated allergic reactions (atopy) with the use of five-percent aqueous mafenide acetate solution and these 17 patients demonstrated rapid resolution of the atopic reaction following administration of an antihistamine and/or discontinuation of the five-percent aqueous mafenide acetate-soaked dressings. Saline or other aqueous topical antimicrobial agents were substituted when five-percent aqueous mafenide acetate-soaked dressings were discontinued. No other adverse reactions were noted in this group of patients.

The use of five-percent aqueous mafenide acetate-soaked dressings has continued to be efficacious, both in the preparation of the burn wound for cutaneous autografting and in the prevention of desiccation of ungrafted granulation tissue. In addition, five-percent aqueous mafenide acetate solution is most helpful in preventing desiccation or premature bacterial colonization of meshed cutaneous autografts. The dressings over such meshed autografted skin can be left in place for an average of three days, allowing development of good adherence of the autografts prior to the first dressing change. The efficacy and the low incidence of adverse side effects speaks for continued use of this solution.

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DAOG6970	86 10 01	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62272A	3S162772A874	AD	163
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY87-01		

11. TITLE *(Precede with Security Classification Code)*
(U) Studies of the Neuroendocrine Abnormalities in Burn Injury

12. SUBJECT AREAS
06 05 Clinical Medicine 06 15 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
79 10	CONT	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY
a. DATE EFFECTIVE
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
86	1.7	118
87	1.7	120

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: VAUGHAN, G M
d. TELEPHONE NUMBER *(include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(include area code)*: 512-221-5416
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(if available)* g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Pineal; (U) Indoles; (U) Catecholamines; (U) Volunteers; (U) Lab Animals: (U) Hamsters; (U) RAII

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23. (U) To characterize sympathetic control of pineal activity in burned soldiers.

24. (U) To develop an animal model of the pineal melatonin response to adrenergic activity.

25. (U) 8510 - 8609. The nocturnal rise in melatonin synthesis, apparently in all mammals, results from beta-adrenergic stimulation of the pineal. In humans, there is a lack of sensitivity to such a stimulus during the daytime. In order to determine whether Sprague-Dawley rats or Syrian hamsters have varying day-night sensitivity, the in vitro response of melatonin synthesis was assessed by incubation of individual whole pineal galdns for four hours without or with different concentrations of isoproterenol in the medium. Pineals were taken either at the end of the 14-hour light phase (day) or at 6-1/2 hours into the 10-hour dark phase (night) after a 30-minute exposure of the animals to light just before sacrifice at night. The response was dose-related and greater at night than at the end of the light phase in rats, but was absent at the end of the light phase in Syrian hamsters. Syrian hamster pineals taken at night responded. Unresponsiveness of Syrian hamster pineals during the day may explain previous failure of isoproterenol administration to stimulate pineal melatonin content in this species. Further, in vivo experiments showed that in Syrian hamsters a subcutaneous injection of isoproterenol at the end of the light phase did not elevate pineal melatonin content

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE

measured two hours later, whereas isoproterenol injected at six hours into the dark phase did (Syrian hamsters were kept in light from 15 minutes before the injection at night). Thus, Syrian hamsters with daytime sensitivity would appear to constitute a better model than does the rat for relevance to the control of human melatonin synthesis.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN BURN INJURY: Nyctohemeral Rhythm in Melatonin Response to Isoproterenol in Syrian Hamsters

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

George M. Vaughan, MD, Lieutenant Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

ABSTRACT

PROJECT NUMBER: 3S162772A874-00, APPLIED RESEARCH

PROJECT TITLE: STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN BURN INJURY: Nyctohemeral Rhythm in Melatonin Response to Isoproterenol in Syrian Hamsters

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: George M. Vaughan, MD, Lieutenant Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

The in vitro response of melatonin synthesis was assessed by incubation of individual whole pineal glands for four hours without or with different concentrations of isoproterenol in the medium. Pineals were taken either at the end of the 14-hour light phase (day), or at 6-1/2 hours into the 10-hour dark phase (night) after a 30-minute exposure of the animals to light just before sacrifice at night. The response was greater in pineals taken at night than in those taken at the end of the light phase in rats, but was absent at the end of the light phase in Syrian hamsters. Hamster pineals taken at night responded, though higher isoproterenol concentrations were required than in the rats. An in vivo response of pineal melatonin content to isoproterenol (1 mg/kg subcutaneously) or saline injected two hours before sacrifice was assessed in hamsters maintained in light following injection. After injection at the end of the light phase, mean pineal melatonin was no different between groups given saline or isoproterenol. Following injection at 15 minutes after novel onset of light beginning 6-1/4 hours into the dark phase, mean pineal melatonin was 6.7-fold higher ($P < 0.001$) two hours after isoproterenol than after saline, the level after saline being similar to the low level present at the time of injection.

Unresponsiveness of hamster pineals during the day may explain previous failure of isoproterenol administration to stimulate pineal melatonin content in this species. The Syrian

hamster pineal becomes sensitive to beta-adrenergic stimulation at night.

BURNS
MELATONIN
ISOPROTERENOL
SYRIAN HAMSTER

NYCTOHEMERAL RHYTHM IN MELATONIN RESPONSE TO ISOPROTERENOL IN SYRIAN HAMSTERS

INTRODUCTION

Because excessive sympathetic activity occurs after burn injury and melatonin synthesis in humans and other mammals is controlled by beta-adrenergic input into the pineal, it will be important to study the significance of melatonin in burn injury. Since Syrian hamsters are like humans in that their pineals appear to be insensitive to adrenergic influence during the daytime, a hamster model of pineal function is now assessed with respect to isoproterenol sensitivity. Pineal melatonin (MEL) synthesis in rats rises in response to beta-adrenergic agonists applied *in vitro* or administered *in vivo* during the day (1-5).

In these experiments, isoproterenol (ISO) was commonly used as the beta-agonist because its action is not influenced by the nerve ending uptake mechanism (5). In contrast to the studies in rats, injection of ISO into Syrian hamsters (with intact or denervated pineals) during the day did not increase pineal melatonin content (6). These results seem incongruent with the previously shown ability of injections of the beta-antagonist propranolol to block the normal *in vivo* nocturnal surge of

---

[1]Klein DC, Weller JL, and Moore RY: Melatonin metabolism: neural regulation of pineal serotonin: acetyl coenzyme A N-acetyltransferase activity. Proc Soc Acad Sci (USA) 68:3107-3110, 1971.

[2]Deguchi T and Axelrod J: Control of circadian change of serotonin N-acetyltransferase activity in the pineal organ by the beta-adrenergic receptor. Proc Natl Acad Sci (USA) 69:2547-2550, 1972.

[3]Brownstein M, Saavedra JM, and Axelrod J: Control of pineal N-acetylserotonin by a beta adrenergic receptor. Mol Pharmacol 9:605-611, 1973.

[4]Axelrod J: The pineal gland: a neurochemical transducer. Science 184:1341-1348, 1974.

[5]Parfitt AG and Klein DC: Sympathetic nerve endings in the pineal gland protect against acute stress-induced increase in N-acetyltransferase (EC 2.3.1.5.) activity. Endocrinology 99:840-851, 1976.

[6]Lipton JS, Petterborg LJ, Steinlechner S, and Reiter RJ: *In vivo* responses of the pineal gland of the Syrian hamster to isoproterenol or norepinephrine. In The Pineal and Its Hormones. Reiter RJ (ed). New York: Alan R. Liss, Inc, 1982, pp 107-115.

melatonin content in the Syrian hamster pineal (7). However, the results in the hamster could be explained by a day/night difference of pineal end-organ sensitivity to the beta-adrenergic action of the endogenous sympathetic neurotransmitter if sensitivity to a beta-agonist could be demonstrated at night when the sympathetic nerves to the pineal ordinarily mediate the nocturnal surge of melatonin synthesis (8-9). The purpose of the present study was to determine whether the pineal melatonin response to ISO differs between the end of the light phase and 6 1/2 hours into the dark phase.

MATERIALS AND METHODS

In all experiments, adult male animals were adapted for more than two weeks to a light cycle with darkness 2000 to 0600 hours and given standard rodent chow and water ad libitum. When the animals were in light, the light intensity was 75 foot-candles. Each experiment was done on a different night.

In experiment 1, Sprague Dawley rats and Syrian hamsters were sacrificed by guillotine at the end of the light phase (2000 hours) or at 0230 hours and the pineals were taken for incubation. For the sacrifice at 0230 hours, the animals were brought into the light 30 minutes prior to sacrifice in order to lower *in vivo* pineal melatonin production acutely to near-daytime levels (10). Pineals were incubated individually as described (11) in 1 ml Minimal Essential Medium containing 10-percent fetal calf serum at pH 7.4 and 37° F under an atmosphere of 95-percent oxygen and five-percent carbon dioxide, without or with ISO at one of several tenfold different concentrations ranging from 10^{-9} to 10^{-5} M for pineals taken at 2000 hours or from 10^{-8} to 10^{-6} M for those

---

[7]Lipton JS, Petterborg LJ, and Reiter RJ: Influence of propranolol, phenoxybenzamine, of phentolamine in the *in vivo* nocturnal rise of pineal melatonin levels in the Syrian hamster. *Life Sci* 28:2377-2382, 1981.

[8]Bowers CW and Zigmond RE: Electrical stimulation of the cervical sympathetic trunks mimics the effects of darkness on the activity of serotonin:N-acetyltransferase in the rat pineal. *Brain Research* 185:435-440, 1980.

[9]Vaughan GM and Reiter RJ: Pineal dependence of the Syrian hamster's nocturnal serum melatonin surge. *J Pineal Res* 3:9-14, 1986.

[10]Rollag MD, Panke ES, Trakulrungsi W, Trakulrungsi C, and Reiter RJ: Quantification of daily melatonin synthesis in the hamster pineal gland. *Endocrinology* 106:231-236, 1980.

[11]Vaughan GM, Lasko J, Coggins SH, Pruitt BA Jr, and Mason AD Jr: Rhythmic melatonin response of the Syrian hamster pineal gland to norepinephrine *in vitro* and *in vivo*. *J Pineal Res* 3:235-249, 1986.

taken at 0230 hours. There were five to six pineals in each dose group. At the end of the four-hour incubation, melatonin (MEL) was determined in the medium, and in the pineal glands after sonication (Figures 1 and 2). The least detectable MEL was 500 pg/ml medium (rat), 50 pg/ml medium (hamster), and 10 pg/pineal. MEL was undetectable in media incubated without a pineal and with ISO at the highest concentration. For graphic purposes, best fit curves were drawn from a three-parameter exponential regression of the MEL data against ISO concentration, which allows a sigmoid plot with respect to log concentration, and data were analyzed by t-tests with the Bonferroni correction for multiplicity of comparisons, analyses of covariance, and in one case a two-way analysis of variance (12).

In experiment 2, hamsters were injected subcutaneously at the end of the light phase (2000 hours) with 0.1 ml physiologic saline or ISO (1 mg/kg) and then maintained in light for two hours until sacrifice when the pineal was taken for determination of melatonin (Figure 3). Means were compared with a t-test.

In experiment 3, five hamsters were sacrificed in the dark at 0215 hours under dim red light (two 25-watt incandescent globes, each with a Kodak No. 1A filter). At 0215 hours, 31 hamsters were brought into a lighted room for sacrifice at times during the next hour in order to determine when pineal melatonin content reached daytime levels. For graphic display (Figure 3), animals were grouped for the time intervals after beginning the light exposure (number of animals): 0-5 minutes (2), 5-10 minutes (3), 10-15 minutes (4), 15-20 minutes (3), 20-30 minutes (7), 30-40 minutes (9), and 40-50 minutes (3). The data were plotted as mean pineal melatonin and mean time of sacrifice. A best-fit decay line was obtained by a single three-parameter exponential regression of the individual data.

In experiment 4, groups of uninjected hamsters were sacrificed in the dark at 0215 hours or two hours later. Three other groups were brought into a lighted room at 0215 hours; one was sacrificed 15 minutes later at 0230 hours, and the other two were injected at 0230 hours with 0.1 ml of either saline or ISO (1 mg/kg) and maintained in light until sacrifice two hours later. The pineals were saved for assay of melatonin (Figure 3), and means of groups sacrificed in the light were compared with a Bonferroni-corrected t-test.

---

[12]Dixon WJ: BMDP Statistical Software. Berkeley: University of California Press, 1983.

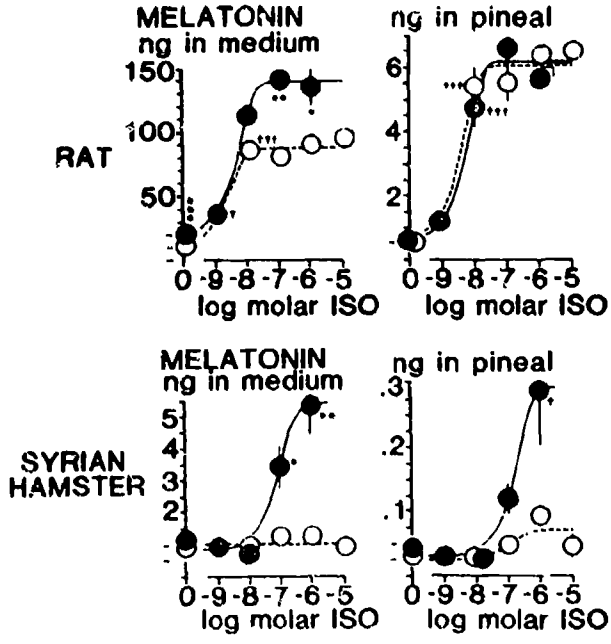


FIGURE 1. Melatonin (± standard error) in the medium of pineal after a 4-hour incubation of individual pineals in 1 ml medium without (zero abscissa point) or with various concentrations of isoproterenol (ISO). Pineals were taken at the end of the light phase (2000 hours, open circles) or 6-1/2 hours after the beginning of the dark phase (0230 hours) just after 30 minutes exposure of the animals to light (closed circles). *P < 0.05, **P < 0.01 vs. 2000-hour group at the same ISO concentration, +P < 0.05, +++P < 0.001 vs. respective group with zero ISO.

In experiment 5, groups of hamsters were sacrificed with a protocol the same as that in experiment 4 except that a group sacrificed in the dark at 0215 hours was not included.

In experiments 2, 4, and 5, each group consisted of seven or eight hamsters. In all experiments, pineals were frozen for later radioimmunoassay of melatonin (13).

---

[13]Vaughan GM, Taylor TJ, Pruitt BA Jr, and Mason AD Jr: Pineal function in burns: melatonin is not a marker for general sympathetic activity. J Pineal Res 2:1-12, 1985.

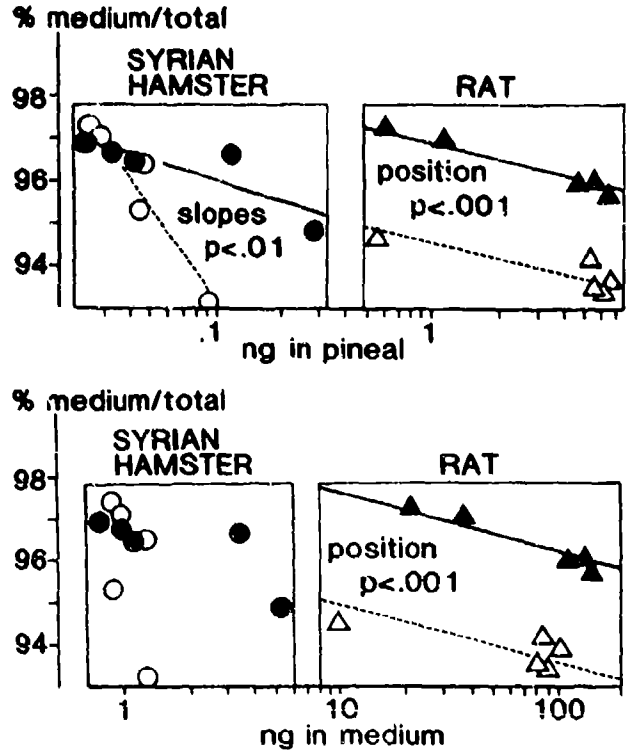


FIGURE 2. Mean proportion of melatonin in the medium (from the data in Figure 1) expressed as a function of the amount of melatonin in the pineal after incubation. The total refers to the combined amount of melatonin in the medium and pineal. Open symbols represent day pineals (2000 hours), and closed symbols night pineals (0230 hours). The difference between day and night slopes is indicated for the hamsters. While the day and night slopes were not significantly different for the rats, the ordinate position of the night values was higher.

RESULTS

Figure 1 (upper panels) shows that after four hours incubation of rat pineals with ISO, MEL content in the medium (though responding to ISO in both day and night experiments) was more elevated for pineals taken at 0230 hours (night) than for those taken at 2000 hours (day). No difference between day and night was seen for MEL remaining in the incubated pineals. A two-way analysis of variance (not shown) compared medium MEL between unstimulated (zero ISO) and stimulated (pool of ISO 10^{-7} and 10^{-6} M) pineals, each with respect to the two times of collection of the pineals. As expected, the main effects of

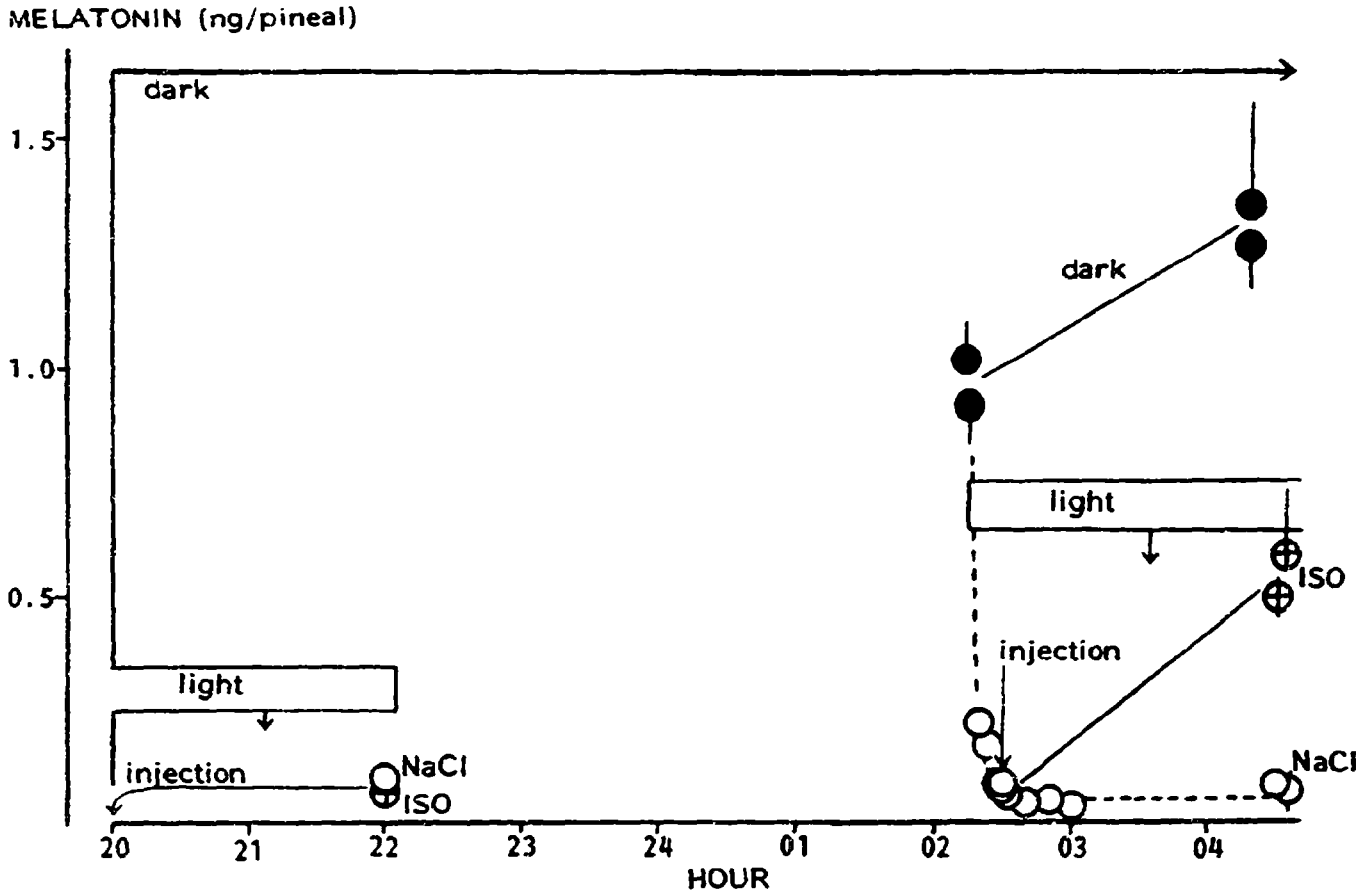


FIGURE 3. Pineal melatonin (mean ± standard error) in Syrian hamsters after subcutaneous injection of isoproterenol (ISO) or saline vehicle (NaCl). Hamsters injected at 2000 hours were kept in a prolonged light phase only on the night of the experiment. Those exposed to light at 0215 hours had not been exposed to light earlier that night and not at all during previous nights (10-hour dark phase). Those injected at 0230 hours had not been injected previously. Groups sampled in light between 0200 and 0300 hours and those sampled in darkness received no injections.

ISO and time of collection were significant. In addition, the significant interaction ($P < 0.01$) indicated that for rat pineals the stimulation of MEL in the medium in the presence of ISO was different (greater) at night from that at the end of the day.

Figure 1 (bottom panels) shows that hamster pineals taken at 2000 hours did not respond to ISO, in that none of the medium or pineal MEL mean values for groups with ISO significantly differed from those of respective controls without ISO. However, medium and pineal MEL for hamster pineals taken at 0230 hours rose with ISO exposure. Even at night, hamster pineals were less sensitive to ISO than were rat pineals, in that the minimal dose producing elevation of medium MEL in hamsters and rats differed by two orders of magnitude. In addition, all mean MEL values for rats were more than tenfold greater than the respective ones for hamsters.

Figure 2 shows that for increasing amounts of MEL in the pineal at the end of the incubation, the proportion of MEL in the medium decreased (common slope $P < 0.001$ for either species). Analysis of covariance showed that this relationship was different between night and the end of the day, in that in either species, for a given amount in the pineal, a greater proportion of the total MEL was in the medium (and consequently a lesser proportion in the pineal) at night. For hamsters, the day/night difference in distribution between the medium and pineal was not evident at the lower amounts in the pineal, but this difference was found over the whole range of observed postincubation pineal MEL in the rats.

The results of experiments 2 through 5 are depicted in Figure 3. In experiment 2, ISO injection at the end of the light phase (2000 hours) did not result in pineal melatonin content different from that after saline injection.

In experiment 3, novel light exposure during the dark phase beginning at 0215 hours resulted in pineal melatonin content typical of daytime levels (9) by 15 minutes in light. The calculated decay half-time was 2.2 minutes and the proportional decay constant was -0.31/minutes. Thus, in experiments 4 and 5, the saline or ISO injection was given after 15 minutes of light when pineal melatonin could be expected to be suppressed adequately. Additional uninjected groups (one in both of these experiments) are included (superimposed) in Figure 3 and were sacrificed at the time of injection of the injected groups and again document adequate suppression. Two hours later, in both experiments, ISO-injected hamsters had pineal melatonin content higher ($P < 0.01$) than that after saline or that in the respective group at the time of injection. The mean ($\pm$ standard error) of the combined ISO groups was 546 ($\pm$ 68) and that of the combined saline groups was 81 ($\pm$ 6) pg/gland

($P < 0.001$). The values for hamsters sacrificed in the dark were typical of those during the normal nocturnal surge of pineal melatonin synthesis (9,13).

DISCUSSION

After incubation, the quantities of MEL remaining in the glands represented only three to seven percent of the total amount present after incubation with or without ISO. Furthermore, in ISO-exposed groups with medium MEL content elevated above the level seen in the comparison group without ISO, the amount of MEL remaining in the ISO-exposed glands was not less than that seen in glands incubated without ISO in either species. Thus, the elevation of medium MEL in groups incubated with ISO represents a response of MEL synthesis, not a net loss of intrapineal MEL. It is evident also from another perspective that the amount of MEL in the medium (in nanogram quantities) represents MEL synthesized during incubation, because even in the groups incubated without ISO, the mean amounts remaining in the pineal were the same as (hamsters) or in excess of (rats) the usual daytime pineal MEL content in glands removed without subsequent incubation in this laboratory (13).

For rats, the response to ISO, present at the end of the light phase, was even greater at night. For hamsters, a significant response occurred only in the pineals taken during the night, with no response of end-light-phase pineals even at an ISO dose two orders of magnitude higher than the lowest dose producing a response in medium MEL from pineals taken during the night. Whether even higher doses of ISO would stimulate daytime or further stimulate nighttime pineals from hamsters was not assessed. Also, since only one incubation time (four hours) was used, it was not assessed whether a time delay in response to ISO contributed to the observed reduction in response at 2000 hours (versus that at 0230 hours) in either species. There is evidence in the rat that the pineal N-acetyltransferase (NAT) responses to injected ISO (2) and to electrical stimulation of the cervical sympathetic trunk (14) are delayed during the day as compared to the night. However, even those daytime responses were evident by two hours.

The reduced sensitivity of hamster pineals (higher doses of ISO required for a response than in rat pineals) and the lower overall MEL values for hamster incubations might have resulted from an effect of light exposure of the animals (including the

---

[14]Bowers CW and Zigmond RE: The influence of the frequency and pattern of sympathetic nerve activity on serotonin N-acetyltransferase in the rat pineal gland. J Physiol 330:279-296, 1982.

30-minute exposure at night) prior to obtaining the pineals. Though it has not yet been adequately tested, we must consider the hypothesis that light exposure itself may have produced a signal in hamsters resulting in less ability of their pineals to respond subsequently, during incubation, to the residual endogenous neurotransmitter (without ISO) and exogenously added ISO. Such an effect in the hamsters (absent or less marked in the rats) might be suspected, because in the conditions of the present study, the highest in vitro intrapineal MEL content achieved at night for rats (about 6 ng) was higher than the normal in vivo peak nocturnal pineal MEL content (about 2 ng); whereas, for hamsters, the highest observed in vitro value (0.3 ng) was lower than the usual mean in vivo nocturnal peak (about 1 ng in this species). The in vivo values (13) were obtained without nocturnal exposure of the animals to light. Other recent experiments (11) have also demonstrated nocturnal responsiveness and daytime unresponsiveness to norepinephrine in Syrian hamster pineals; the somewhat smaller pineal weight in hamsters (25 percent of that in rats) did not fully account for the much greater difference of in vitro norepinephrine-stimulated MEL production between species after light exposure.

The altered distribution of MEL between gland and medium after incubation (propensity for a greater proportion to be in the medium for a given amount in the pineal at night than at the end of the day) in both species is not yet understood. However, one explanation might be that there is a mechanism to enhance transport of MEL out of the pineal gland and that this mechanism is more active at night. Whether these in vitro observations reflect a more active in vivo MEL secretory process at night is not yet known.

The end of the light phase was chosen for measuring daytime sensitivity, because in rats, this was the time of greatest sensitivity of pineal NAT stimulation by ISO during the light phase (15).

In the present studies, the in vitro pineal response of MEL synthesis to beta-adrenergic stimulation for four hours h was greater 6-1/2 hours into the dark phase than at the end of the light phase in rats, and it was absent at the end of the light phase in hamsters under the conditions of the present study. Hamster pineals taken at night responded (though with less sensitivity than those of rats). Whether this represents a relative receptor or postreceptor defect for beta-adrenergic stimulation during the day is not yet known. However, a

---

[15]Romero JA and Axelrod J: Pineal beta-adrenergic receptor: diurnal variation in sensitivity. Science 184:1091-1092, 1974.

contributory role for a nyctohemeral difference in receptors is likely in that pineal beta-receptor density was lower during the day than at night in the rat (16).

Whatever the mechanism, the relatively profound *in vitro* unresponsiveness of hamster pineals at the end of the day may explain the present observation of lack of *in vivo* pineal MEL response to ISO injected at that time. Others have also found lack of effectiveness of ISO injections during or near the end of the light phase in hamsters (6; Reiter, personal communication). That hamsters might be sensitive to ISO *in vivo* at night was presaged by Steinlechner and others (17) who found that ISO given to hamsters in the dark at night (while pineal melatonin was still high) prevented the fall in pineal melatonin that otherwise accompanies light exposure. We now have shown that when pineal melatonin is first made low by light at night during the time of the normal nocturnal melatonin surge in darkness, subcutaneously injected ISO is capable of stimulating pineal melatonin content from low to higher levels, an *in vivo* result confirming the effect we found *in vitro*. Elevation of pineal melatonin also one to three hours h after intraperitoneal ISO injection in light exposed hamsters at night has recently been found (Reiter, Vaughan, Oaknin, *et al*, unpublished results). With blockade of neuronal uptake by desipramine, a similar response to norepinephrine can be demonstrated in hamsters under these conditions (11).

Thus, it is clear that the Syrian hamster develops responsiveness to beta-adrenergic stimulation during the time at night when its pineal normally receives melatonin-stimulatory signals from sympathetic nerves innervating the gland but remains unresponsive at other times. That the pineal melatonin level reached after *in vivo* injection of an adrenergic agent did not reach the normal nighttime level in this and the quoted studies may have resulted from inability to produce local concentrations of agent at the pinealocyte from the circulation after injection that are equivalent to those possible from normal local neuronal transmission in the gland, or from induction of insensitivity to beta-agonists by the light exposure used to interrupt the normal

---

[16]Esquifino AI, Craft CM, Champney TH, *et al*: Pineal melatonin levels and beta-receptor density: changes associated with puberty in the male rat. *In* The Pineal Gland: Endocrine Aspects. Brown GM and Wainwright S, eds. New York: Pergamon Press, 1985, pp 139-144.

[17]Steinlechner S, King TS, Champney TH, *et al*: Pharmacological studies on the regulation of N-acetyltransferase activity and melatonin content of the pineal gland of the Syrian hamster. *J Pineal Res* 2:109-119, 1985.

neurotransmitted signals, or some other factor. Plans are being made to assess adrenergic receptor function under these conditions.

PRESENTATIONS/PUBLICATIONS

Vaughan GM: Human melatonin in physiologic and diseased states: neural control of the rhythm. Presented to the Conference on Melatonin in Humans, Vienna, Austria, 7-9 November 1985 (to be published in J Neural Transm in 1986).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DAOG6972	2. DATE OF SUMMARY: 86 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 85 10 01	4. KIND OF SUMMARY: D	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DISB'N INSTR'N: CX	9. LEVEL OF SUM / A. WORK UNIT:

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BD	301
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP	FY87-01		

11. TITLE *(Precede with Security Classification Code)* (U) Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injury

12. SUBJECT AREAS
06 05 Clinical Medicine 06 13 Microbiology 06 15 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
76 10	CONT	DA	C

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED / APPROVED BY [signature]

a. DATE EFFECTIVE	b. CONTRACT/GRANT NUMBER	c. TYPE	d. AMOUNT	e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
86	0.5	254
87	0.5	256

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: MC MANUS, A T
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-3411
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)* g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Pseudomonas; (U) Klebsiella; (U) Staphylococcus; (U) Wound Infection; (U) Antibiotic Resistance;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

22. (Continued) (U) Sepsis; (U) Topical Chemotherapy; (U) Volunteers; (U) Lab Animals: (U) Rats; (U) Guinea Pigs; (U) Mice; (U) RAII

23. (U) Burns constitute a large component of military injuries sustained in combat. Military relevance of this research lies in the fact that infection and ensuing sepsis are major problems among burned soldiers. Control of surface infection is a major objective and species of organisms causing sepsis, epidemiology, response of significant species to topical chemotherapy modalities, and relationship of antibiotics to sepsis control are major study areas.

24. (U) Cultures of human wounds, tissues, and body fluids are carried out with precise strain speciation and differentiation being employed. Virulence is assessed in burn wound models which are also used to assess effectiveness of experimental drugs, both topical and systemic.

25. (U) 8501 - 8512. During calander year 1985, microbiologic surveillance was carried out on 190 of the 197 admitted and discharged burn patients. More than 8,000 isolates were recovered. As has been the experience in recent years, relative isolation of Gram-negative organisms continued to be low. The most common Gram-negative isolate was Pseudomonas aeruginosa, but its recovery was less than 30 percent of patients and eight percent of the total flora. Positive blood cultures were recovered from 38 patients with Staphylococcus aureus being the most common offender.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 January 1985 - 31 December 1985

INVESTIGATORS

Albert T. McManus, PhD
Jack R. Henderson, PhD
Timothy E. Lawson, Staff Sergeant
Charles H. Guymon, Sergeant
Aldo H. Reyes, Staff Sergeant
Kenneth Hawthorne, Staff Sergeant
Anne Lasko, Sergeant
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Jan 85 through 31 Dec 85

INVESTIGATORS: Albert T. McManus, PhD
Jack R. Henderson, PhD
Timothy E. Lawson, Staff Sergeant
Charles H. Guymon, Sergeant
Aldo H. Reyes, Staff Sergeant
Kenneth Hawthorne, Staff Sergeant
Anne Lasko, Sergeant
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

During calendar year 1985, 188 burned patients were cultured and 9,044 isolates were identified. A relatively low colonization frequency (31 percent) with Gram-negative organisms has continued for the fourth reporting period. This was also reflected in an increase in Gram-positive organisms in blood cultures. Staphylococcus aureus was the most frequent blood pathogen. Antibiotic resistance in Staphylococcus was found to have increased from recent reporting periods. The computerized microbial culture surveillance system has been extended to include infection control and antibiotic usage data bases. This system is being evaluated for its use in predicting infecting organisms from previous sites of colonization and antibiotic usage.

BURN MICROBIOLOGY
PSEUDOMONAS
KLEBSIELLA
STAPHYLOCOCCUS
ANTIBIOTIC RESISTANCE
BLOOD CULTURE
BIOPSY
HUMANS

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY

INTRODUCTION

This report is produced from microbiology data collected for patients admitted during calendar year 1985. Data were collected from admission through disposition. This is the second report that is based on calendar year rather than fiscal year. This change more nearly aligns culture results with the annual research progress report produced by the Clinical Division for the same patient population.

AUTOMATED MICROBIOLOGY DATA BASE

The microbiology data base now contains complete surveillance data for more than 788 burn patient admissions. Epidemiologic use of these data has resulted in several publications. The microbiology data base has been aligned with antibiotic use and infection control data bases. This has improved the utility of the system for prospective use in predicting the antibiotic sensitivity of infecting organisms.

ANTIBIOTIC SENSITIVITY DETERMINATION

The 1985 antibiotic testing panels are presented in Table 1. Bacterial organisms were tested by agar overlay disc diffusion. Broth dilution minimal inhibitory concentrations and minimal bactericidal concentrations were available upon specific request. The protocol for selecting organisms for *in vitro* sensitivities was isolation from blood cultures, predominant organisms in biopsy cultures, predominant organisms in urine cultures with more than 10^5 colony-forming units per milliliter, *Staphylococcus aureus* isolates, *Pseudomonas aeruginosa* isolates, and any other organism requested.

MICROBIAL SURVEILLANCE

The microbial surveillance protocol established in fiscal year 1983 was continued in calendar year 1985 (1). Patients were cultured from wound, sputum, urine, and rectum on admission. Thereafter, sputum and urine were cultured three times per week and stools and wound surfaces twice per week. Patients transferred to the convalescent ward and hospitalized

---

[1]McManus AT, Henderson JR, Lawson TJ, *et al*: Studies of Infection and Microbiologic Surveillance of Infection in Troops with Thermal Injury. *In* US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1985, pp 146-194.

TABLE 1. *In vitro* SENSITIVITY PANELS FOR 1985

Enteric Organisms	Nonenteric Gram-Negative Organisms	Gram-Positive Organisms
1. Amikacin[a,c]	1. Amikacin[a,c]	1. Amikacin[a,c]
2. Gentamicin[a,c]	2. Gentamicin[a,c]	2. Gentamicin[a,c]
3. Tobramycin[a,c]	3. Tobramycin[a,c]	3. Tobramycin[a,c]
4. Ticarcillin[a]	4. Ticarcillin[a]	4. Ticarcillin
5. Mezlocillin[a,c]	5. Mezlocillin[a,c]	5. Mezlocillin[a,c]
6. Piperacillin[a,c]	6. Piperacillin[a,c]	6. Piperacillin[a,c]
7. Moxalactam[a,c]	7. Moxalactam[a,c]	7. Moxalactam[c]
8. Cefotaxime[a]	8. Cefotaxime[a]	8. Cefotaxime
9. Cefoperazone	9. Cefoperazone[a]	9. Cefoperazone
10. Sulfadiazine	10. Cefsulodin[a]	10. Cefsulodin
11. Netilmicin[a]	11. Colistin	11. Sulfadiazine
12. Kanamycin	12. Sulfadizine[a]	12. Methicillin[a]
13. Chloramphenicol	13. Netilmicin	13. Cephalothin[a]
14. Tetracycline	14. Kanamycin	14. Vancomycin[a,c]
15. Cefoxitin[a]	15. Chloramphenicol	15. Kanamycin
16. Cefamandole[a]	16. Tetracycline	16. Chloramphenicol[a]
17. Ampicillin[a]	17. MK0787[b,c]	17. Tetracycline
18. Neomycin	18. Azlocillin[a]	18. Ampicillin
19. Trimethoprim		19. MK0787[b,c]
20. Trimeth and Sulfa		20. Clindamycin[a]
21. Nalidixic Acid		21. Penicillin[a]
22. MK0787[b,c]		22. Erythromycin[a]
23. Streptomycin		23. Streptomycin

[a]Reported daily on daily clinical microbiology report (hard copy).
[b]Experimental drug.
[c]Reported on computer screen from patient data base.

more than 30 days were cultured once per week. Gentamicin-resistant Gram-negative organisms from sputum or stool specimens were screened by plating on MacConkey agar containing gentamicin sulfate (20 micrograms per milliliter).

MICROBIOLOGIC FINDINGS IN BURN PATIENTS

A total of 197 patients admitted in 1985 were cultured. Species isolated and number of patients yielding each species are presented in Table 2. Because of the decreased resistance of the patient population, no organism is considered "normal" flora and all isolated organisms are reported to the physician. A summary of the ten most common isolates is presented in Table 3. The table contains 77.8 percent of the species identified. The relative frequencies of sites of isolation are presented in Figure 1. The relative frequencies of sites of isolation of Gram-negative organisms, Gram-positive organisms, and yeast are shown in Figure 2.

FLORA RECOVERED FROM RESPIRATORY SYSTEM SPECIMENS

A total of 6,666 organisms were recovered from respiratory system specimens. The majority of these were sputum cultures collected in the surveillance program. The ten most frequent species are presented in Table 4, which represents 77 percent of the respiratory isolates. Of particular note is the continued decline of Gram-negative isolates. Pseudomonas aeruginosa was isolated from 23 of the 183 patients cultured. This frequency was not significantly different from calendar year 1984.

FLORA RECOVERED FROM WOUND SURFACE SPECIMENS

A total of 1,126 contact plate surface cultures were taken and 457 isolates were made. This is a significant decrease in isolation frequency from 1984 ($P < 0.01$). Relative frequencies of isolated species are presented in Figure 3. Subsurface flora, as measured by biopsy specimens, was measured in 155 biopsies taken from 33 patients. Organisms were recovered from 21 of the biopsied patients. The ten most common organisms are presented in Table 5. Filamentous fungi remained the principal isolate (50 percent) with Aspergillus being the most common fungal genus. Pseudomonas aeruginosa was recovered from five biopsies taken from two patients. The continued decrease in recovery of wound bacteria is best correlated with the decrease in resistance to topical and parenteral antimicrobial agents. The loss of antagnostic bacterial flora is a reasonable basis for the increased frequency of fungal isolates.

TABLE 2. DISTRIBUTION BY ORGANISM

Organism	Number of Isolates	Number of Patients Colonized	Organism	Number of Isolates	Number of Patients Colonized
Acinetobacter anitratus	22	6	Neisseria lactamicas	1	1
Acinetobacter lwoffii	3	3	Neisseria mucosa	149	62
Aspergillus flavus	1	1	Propionibacterium acnes	2	2
Aeromonas hydrophila	3	3	Proteus mirabilis	201	38
Bacillus	22	24	Proteus vulgaris	2	2
Branhamella catarrhalis	25	6	Pseudomonas aeruginosa	562	53
Candida albicans	221	50	Pseudomonas cepacia	2	2
Candida parapsilosis	6	3	Pseudomonas fluorescens	1	1
Candida rugosa	130	31	Pseudomonas putida	1	1
Candida tropicalis	20	8	Serratia marcescens	159	22
Citrobacter freundii	56	13	Staphylococcus aureus	1,558	140
Citrobacter diversus	3	3	Staphylococcus epidermidis	179	84
Clostridium difficile	1	1	Staphylococcus saprophyticus	51	33
Clostridium hemolyticus	1	1	Alpha Streptococcus	43	21
Corynecbacterium pyogenes	1	1	Beta Streptococcus, Not Group A, B, or D	141	52
Enterobacter aerogenes	125	36			
Enterobacter agglomerans	19	7	Group D Streptococcus, Not Enterococcus	144	71
Enterobacter cloacae	85	30			
Escherichia coli	508	74	Group D Enterococcus	177	61
Haemophilus influenzae	42	10	Nonhemolytic Streptococcus	26	17
Haemophilus parainfluenzae	15	2	Nonhemolytic Streptococcus, Not Group D	951	163
Klebsiella oxytoca	28	15			
Klebsiella pneumoniae	426	87	Streptococcus pneumoniae	30	23
Morganella morganii	35	9	Streptococcus viridans	1,666	174
Mucor species	2	2	True Fungi Species (Other)	50	34
Neisseria gonorrhea	2	1	Yeast Species (Other)	20	10

TOTAL NUMBER OF ISOLATES = 8,127

TOTAL NUMBER OF PATIENTS = 188

TABLE 3. TEN MOST FREQUENT ISOLATES (1985)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Streptococcus viridans	174	92.5	1,666	20.5
Nonhemolytic Streptococcus, Not Group D	163	86.7	951	11.7
Staphylococcus aureus	140	74.4	1,558	19.2
Klebsiella pneumoniae	87	46.3	426	5.3
Staphylococcus epidermidis	84	44.6	179	2.2
Escherichia coli	74	39.3	508	6.3
Group D Streptococcus, Not Enterococcus	71	37.8	144	1.8
Neisseria mucosa	62	32.9	149	1.8
Enterobacter species	61	32.4	177	2.2
Pseudomonas aeruginosa	53	28.2	562	6.9
			6,320	77.8

TOTAL NUMBER OF PATIENTS CULTURED = 188
TOTAL NUMBER OF ISOLATES =

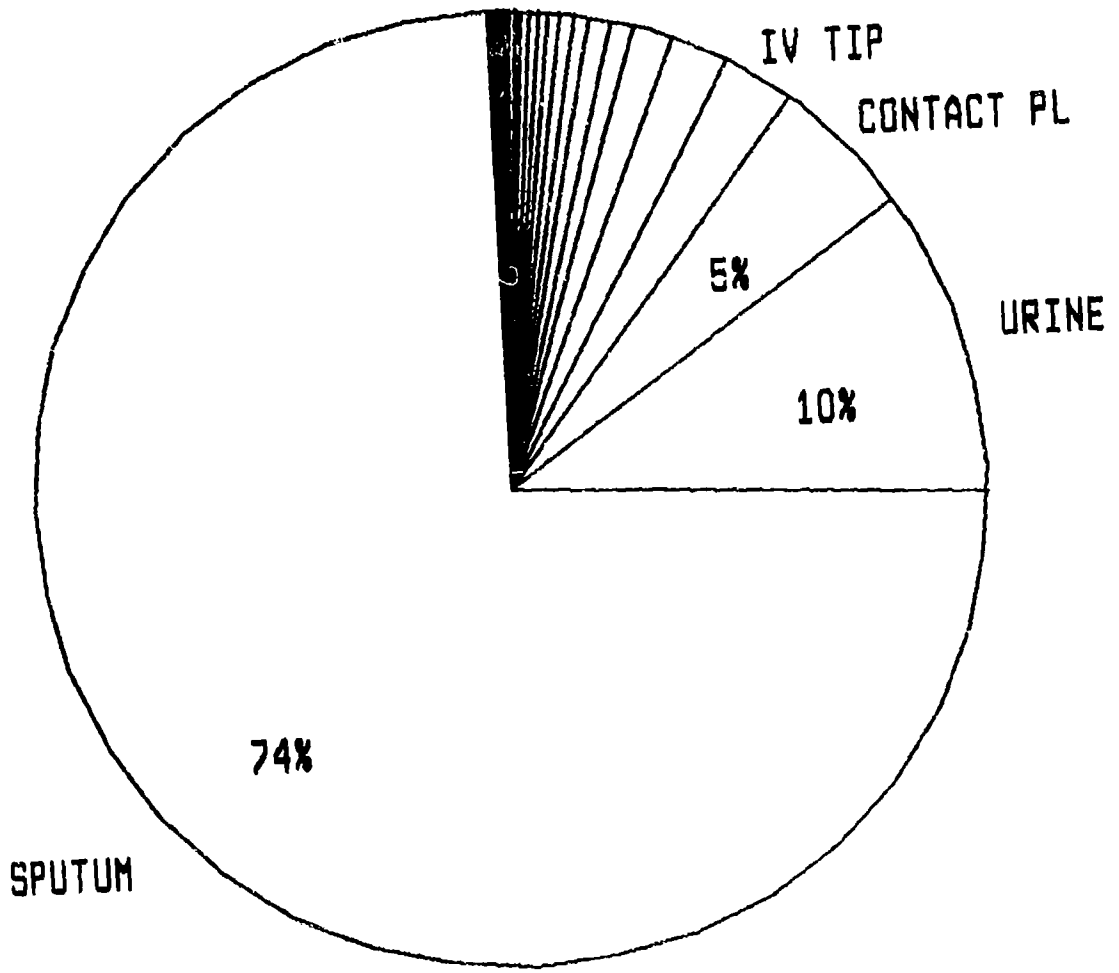


Figure 1. Display of the relative frequency of specimen sources yielding isolates in 1985.

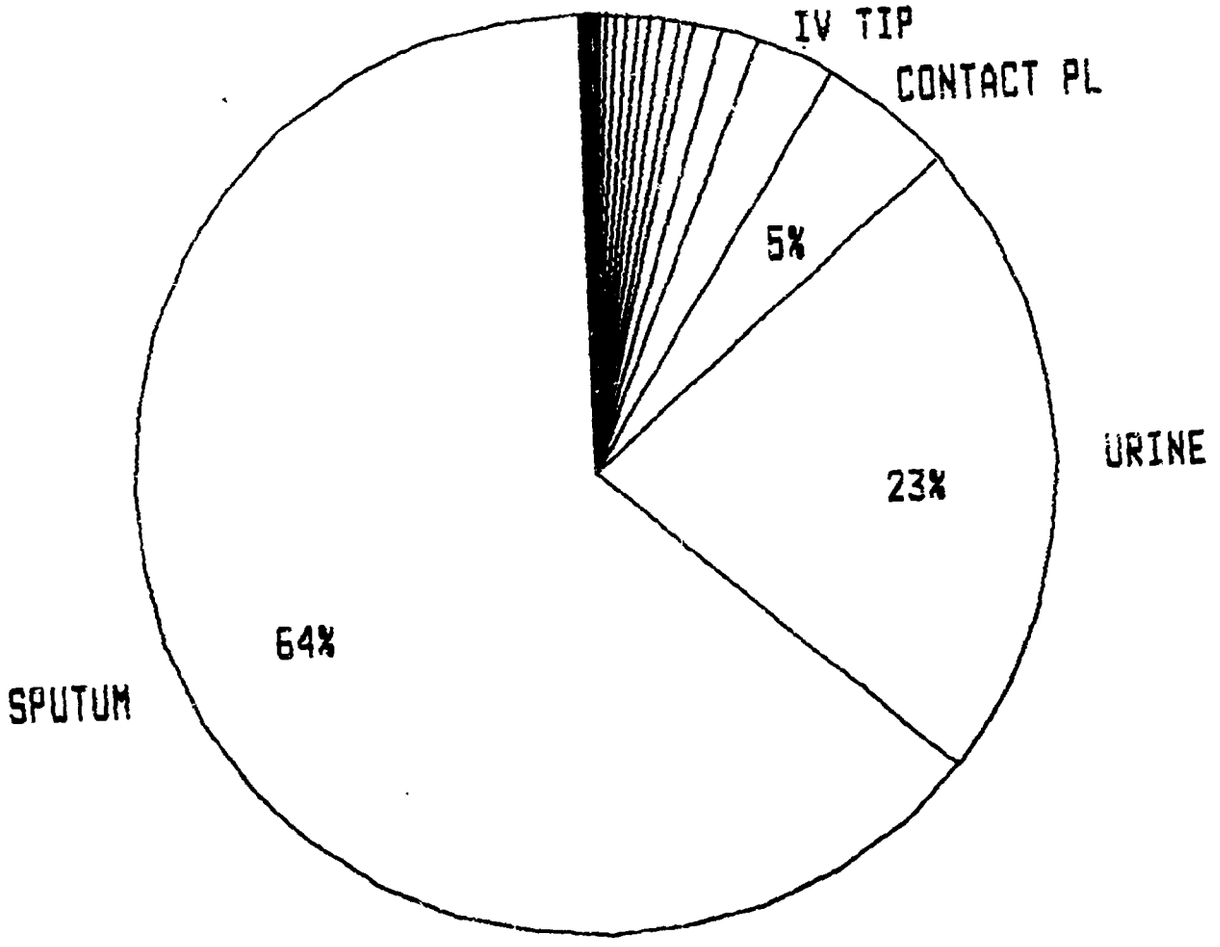


Figure 2a. Display of the relative frequency of specimen sources yielding Gram-negative organisms.

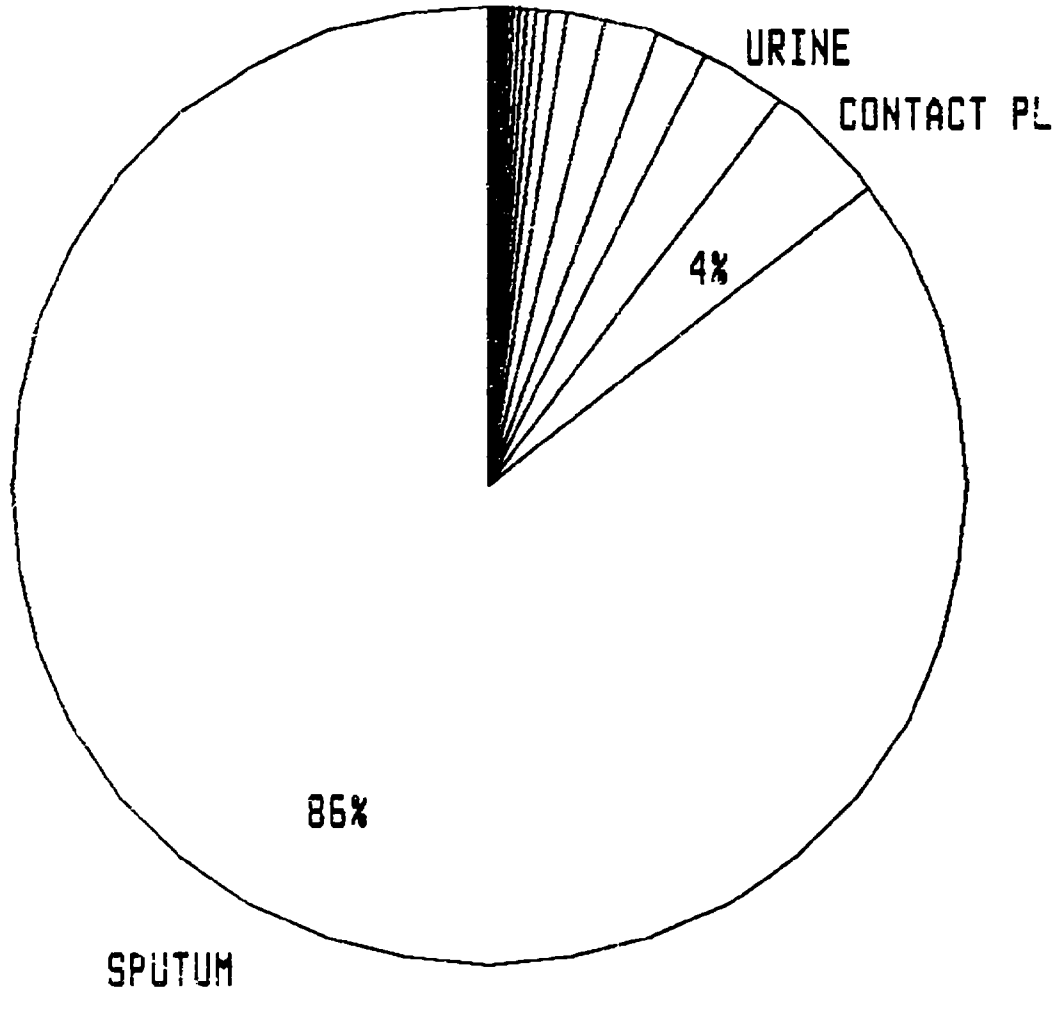


Figure 2b. Display of the relative frequency of specimen sources yielding Gram-positive organisms.

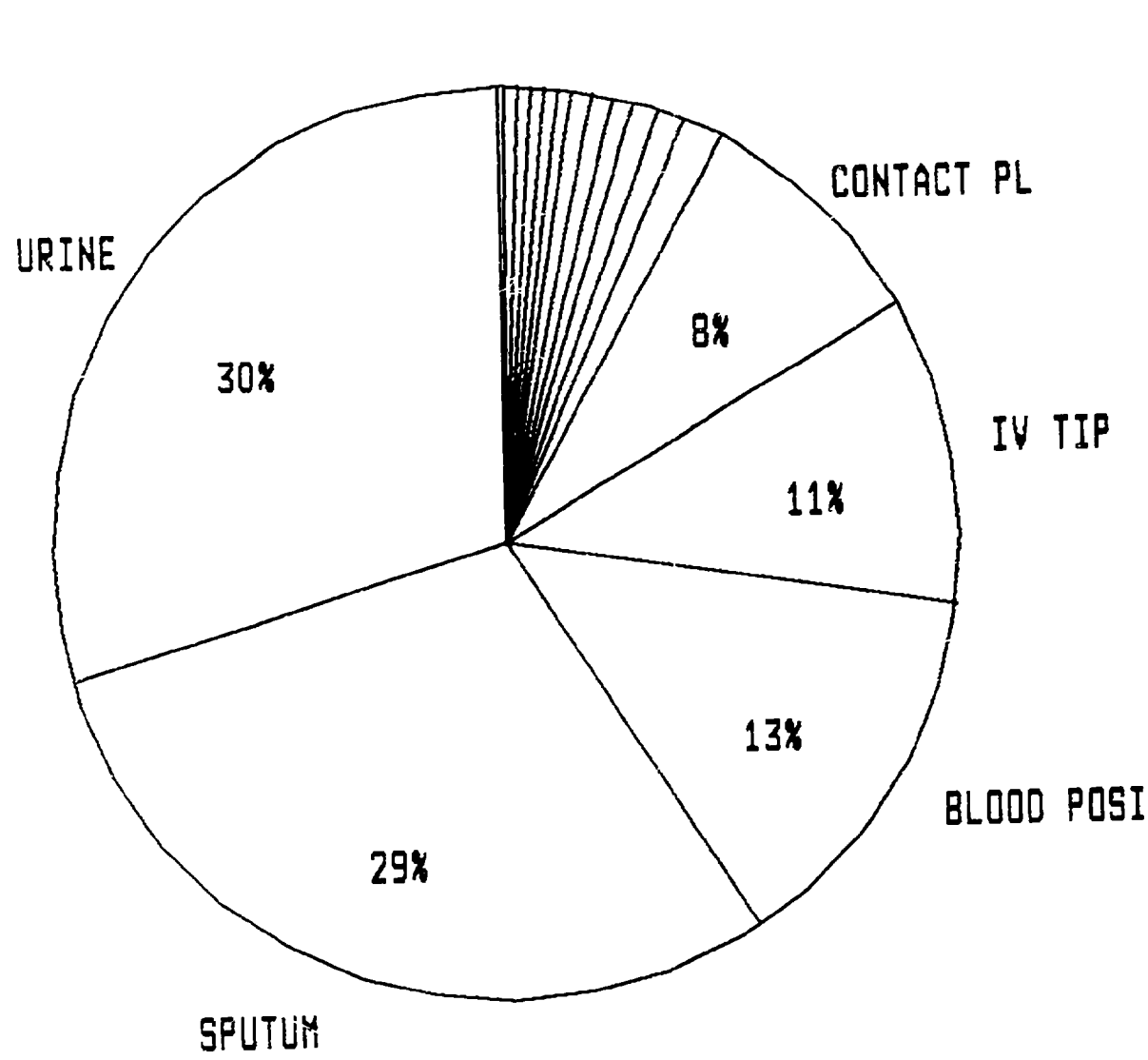


Figure 2c. Display of the relative frequency of specimen sources yielding yeast-like organisms.

TABLE 4. TEN MOST FREQUENT ISOLATES FROM RESPIRATORY SOURCES (1985)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Streptococcus viridans	171	93.4	1,589	25.1
Nonhemolytic Streptococcus, Not Group D	162	88.5	876	13.9
Staphylococcus aureus	120	66.6	1,259	19.9
Group D Streptococcus, Not Enterococcus	67	36.2	137	2.1
Neisseria mucosa	61	33.3	148	2.3
Beta-Hemolytic Streptococcus, Not Group A, B, or D	52	28.4	137	2.2
Staphylococcus epidermidis	45	24.6	78	1.2
Candida albicans	40	21.9	88	1.4
Klebsiella pneumoniae	35	19.1	224	3.5
Escherichia coli	33	18.3	211	3.2
			4,872	77.0

TOTAL NUMBER OF PATIENTS CULTURED = 183
TOTAL NUMBER OF ISOLATES = 6,666

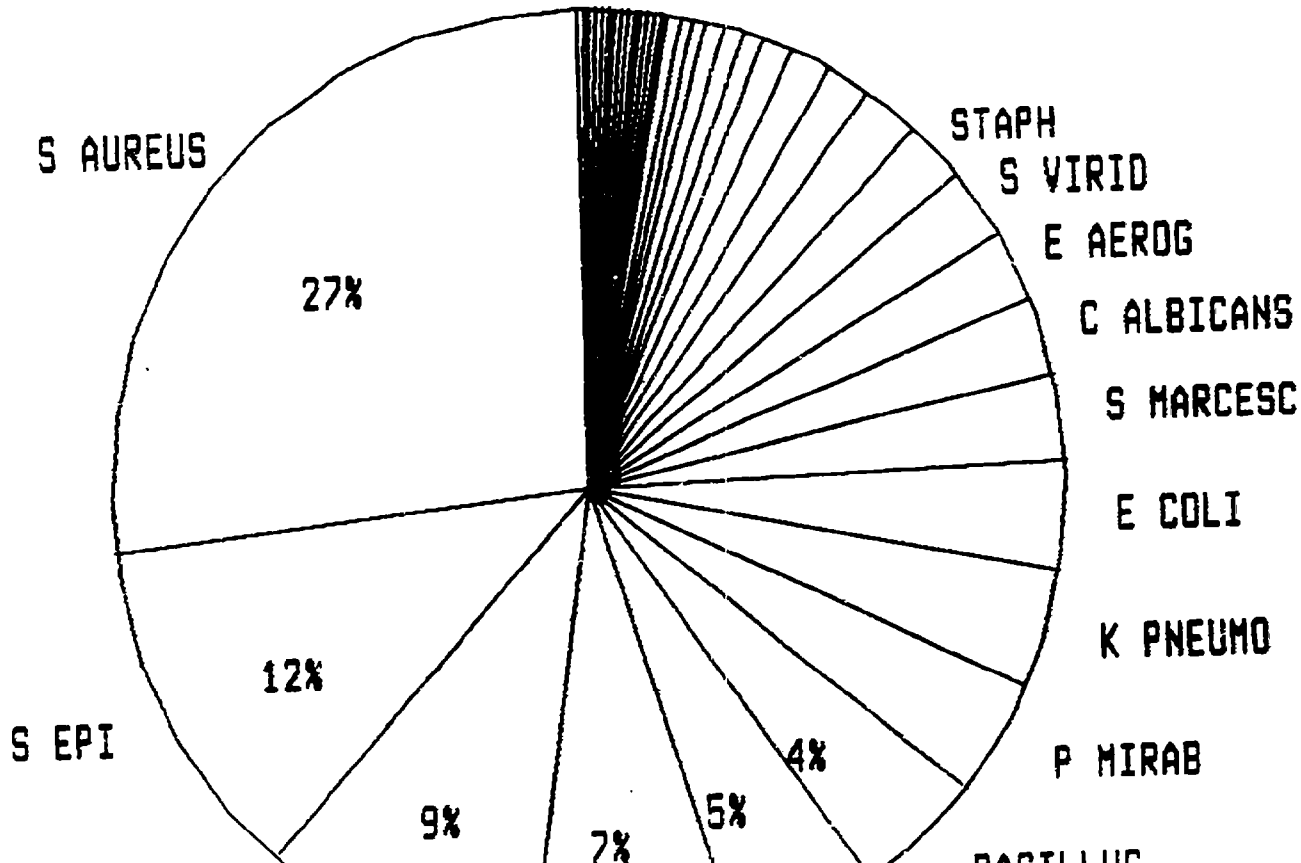


Figure 3. Display of the relative frequency of organism types isolated from surface wound cultures.

TABLE 5. PRINCIPAL ORGANISMS RECOVERED IN BIOPSY SPECIMENS (1985)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Filamentous fungi	16	48.5	35	50.0
Group D Enterococcus	4	12.1	8	11.4
Staphylococcus aureus	4	12.1	5	7.1
Candida rugosa	4	12.1	4	5.7
Enterobacter cloacae	3	9.1	4	5.7
Pseudomonas aeruginosa	2	6.1	5	7.1
Candida albicans	2	6.1	2	2.9
Escherichia coli	2	6.1	2	2.9
Enterobacter aerogenes	1	3.0	1	1.4
Serratia marcescens	1	3.0	1	1.4
			67	95.7

TOTAL NUMBER OF PATIENTS BIOPSIED = 33
TOTAL NUMBER OF ISOLATES = 70
BIOPSIES TAKEN = 155

FLORA RECOVERED FROM URINARY TRACT SPECIMENS

Urine specimens from 120 patients yielded 1,053 isolates. The ten most common species are presented in Table 6. The principal change noted from fiscal year 1983 was a significant ($P < 0.05$) increase in Klebsiella pneumoniae isolates. Escherichia coli isolation frequencies did not change between reporting periods. The top ten organisms isolated from urine specimens with greater than 10^5 colony-forming units per milliliter are presented in Table 7.

FLORA RECOVERED FROM BLOOD CULTURES

Blood cultures were obtained from 119 patients for a total of 1,541 cultures. The principal organisms recovered are listed in Table 8. Positive cultures were obtained from 38 patients and 165 isolates were made from 166 positive cultures. Ninety cases of bacteremia were noted using a case definition of isolation of an organism once or more than once with a 30-day period equaling a case.

Intravenous catheter tips were cultured from 93 patients. Isolations were made from 59 patients and 203 isolates were made. Data are presented in Table 9. These data show an unexpectedly high incidence of contamination.

SUMMARY OF ANTIBIOTIC TESTING

A total of 2,711 bacterial isolates were tested for in vitro sensitivity to antibiotics. A comparison of sources of tested strains is presented in Figure 4. The relative frequency of tested organisms is presented in Figure 5.

Gentamicin resistance was again used as a plasmid surveillance marker. Testing was done on 2,711 isolates. Figure 6 displays the relative frequency of tested organisms. Figure 7 displays the frequency of resistant species. As noted in the US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1985, multiple resistant Enterobacter cloacae were the principal resistant isolates.

Antibiotic sensitivity patterns for the principal bacterial species isolated from blood cultures are presented separately.

Staphylococcus aureus. The sources of Staphylococcus aureus strains tested for in vitro activity are presented in Figure 8. The results of in vitro testing are presented in Table 10. An overall increase in antibiotic resistance compared to fiscal year 1983 was noted. The incidence of methicillin resistant isolates has increased above the previous six reporting periods. The resistant strains are multiply resistant with genes for gentamicin, erythromycin, oxacillin,

TABLE 6. TEN MOST FREQUENT ORGANISMS FROM URINARY SPECIMENS (1985)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Klebsiella pneumoniae	65	34.9	141	15.7
Escherichia coli	58	31.2	124	13.8
Group D Enterococcus	35	18.8	60	6.7
Pseudomonas aeruginosa	34	18.3	102	11.4
Proteus mirabilis	28	15.1	118	13.1
Staphylococcus aureus	24	12.9	36	4.0
Candida albicans	18	9.7	72	8.0
Nonhemolytic Streptococcus, Not Group D	18	9.7	22	2.4
Candida rugosa	16	8.6	28	3.1
Streptococcus viridans	13	6.9	15	1.2
			718	80.0

TOTAL NUMBER OF PATIENTS CULTURED = 186
TOTAL NUMBER OF ISOLATES = 898

TABLE 7. TEN MOST FREQUENT ORGANISMS FROM URINARY SPECIMENS WITH > 10^5 CFU (1985)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Escherichia coli	34	41.5	49	14.1
Klebsiella pneumoniae	31	37.8	50	14.4
Pseudomonas aeruginosa	22	26.8	63	18.1
Proteus mirabilis	12	14.6	30	8.6
Group D Enterococcus	12	14.6	22	6.3
Candida albicans	9	18.3	27	7.8
Nonhemolytic Streptococcus, Not Group D	9	11.0	11	3.2
Candida rugosa	8	9.8	8	2.3
Enterobacter cloacae	5	6.1	6	1.7
Morganella morganii	5	6.1	6	1.7
			272	78.2

TOTAL NUMBER OF PATIENTS CULTURED = 82
TOTAL NUMBER OF ISOLATES = 348

TABLE 8. PRINCIPAL ORGANISMS FOUND IN BLOOD CULTURES (1985)

Organism	Number of Patients	% Patients	Number of Cases	% Cases	Number of Isolates	% Total Isolates
Staphylococcus aureus	18	15.0	18	24.7	47	28.5
Candida rugosa	7	5.8	7	9.6	27	16.4
Staphylococcus epidermidis	7	5.8	7	9.6	11	6.7
Candida albicans	5	4.2	5	6.8	22	13.3
Klebsiella pneumoniae	5	4.2	5	6.8	10	6.1
Escherichia coli	4	3.3	4	5.5	8	4.8
Staphylococcus saprophyticus	4	3.3	4	5.5	5	3.0
Pseudomonas aeruginosa	2	1.7	2	2.7	6	3.6
Serratia marcescens	2	1.7	2	2.7	3	1.8
Enterobacter cloacae	2	1.7	2	2.7	3	1.8
			56	76.7	141	85.5

TOTAL NUMBER OF PATIENTS CULTURED = 119
TOTAL NUMBER OF ISOLATES = 165
TOTAL NUMBER OF CULTURES = 1,053
TOTAL NUMBER OF PATIENT POSITIVES = 38

TABLE 9. TEN MOST FREQUENT ORGANISMS FROM INTRAVENOUS CATHETERS (1985)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Staphylococcus aureus	18	18.9	30	14.8
Group D Enterococcus	13	13.7	16	7.9
Staphylococcus epidermidis	13	12.6	14	6.9
Candida rugosa	12	12.6	27	13.3
Pseudomonas aeruginosa	11	11.6	17	8.4
Klebsiella pneumoniae	9	9.5	21	10.3
Nonhemolytic Streptococcus, Not Group D	9	9.5	9	4.4
Streptococcus viridans	9	9.5	9	4.4
Candida albicans	8	8.4	12	5.9
Serratia marcescens	4	4.2	11	5.4
			166	81.8

TOTAL NUMBER OF PATIENTS CULTURED = 95
TOTAL NUMBER OF ISOLATES = 203

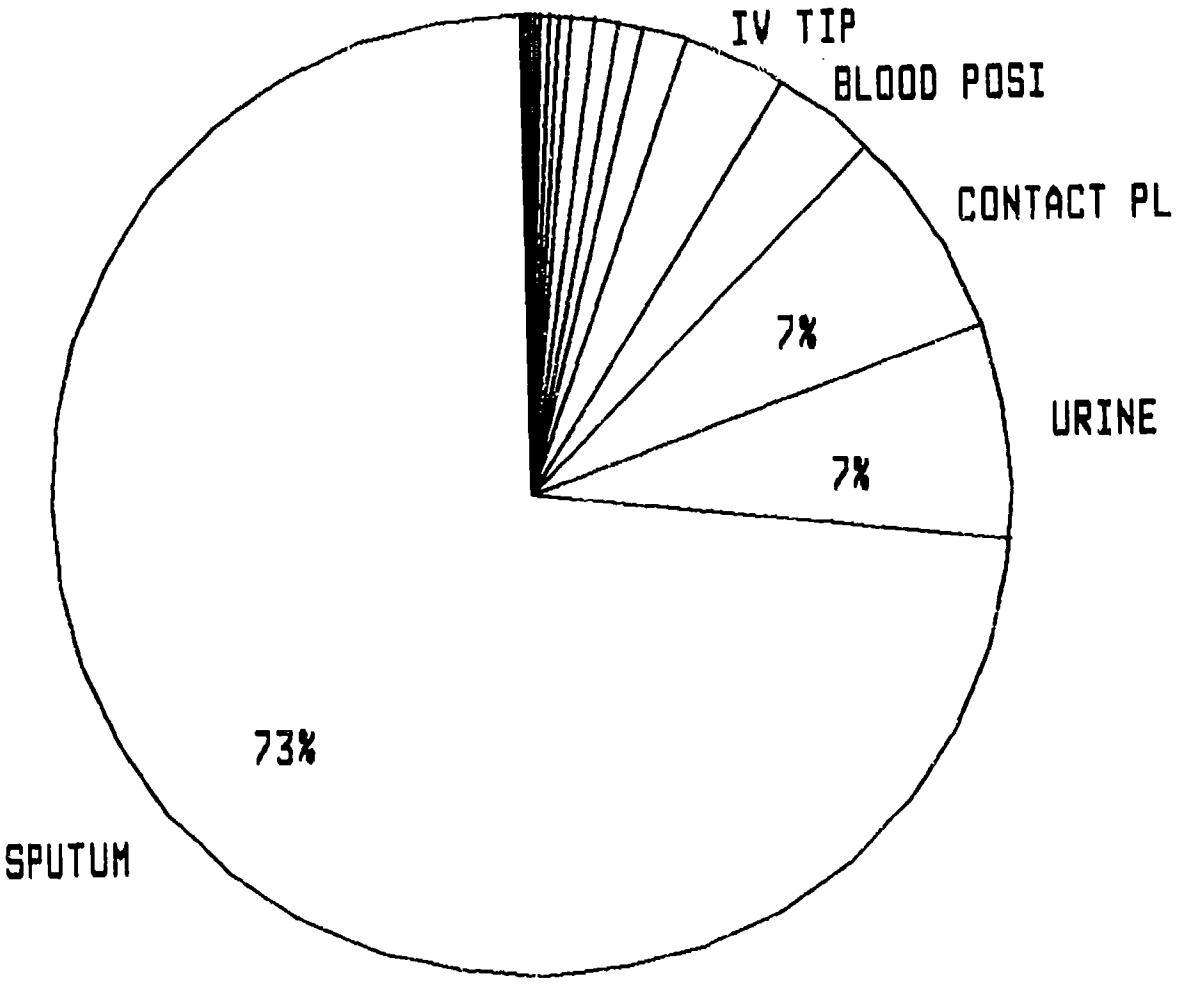


Figure 4. Display of the relative frequency of sources yielding organisms tested for in vitro sensitivity to antibiotics in 1985.

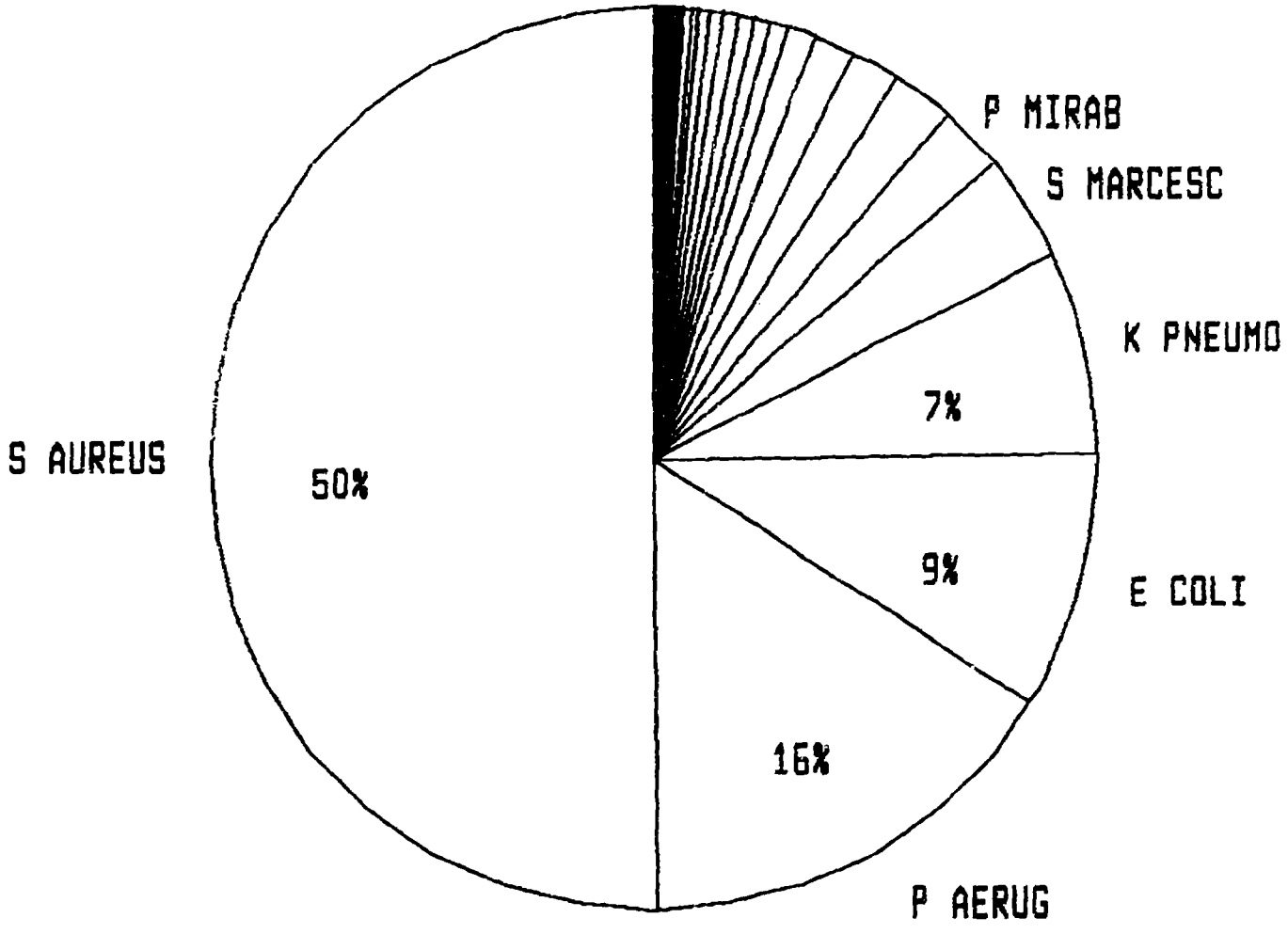


Figure 5. Display of the relative frequency of organisms tested for *in vitro* sensitivity to antibiotics in 1985.

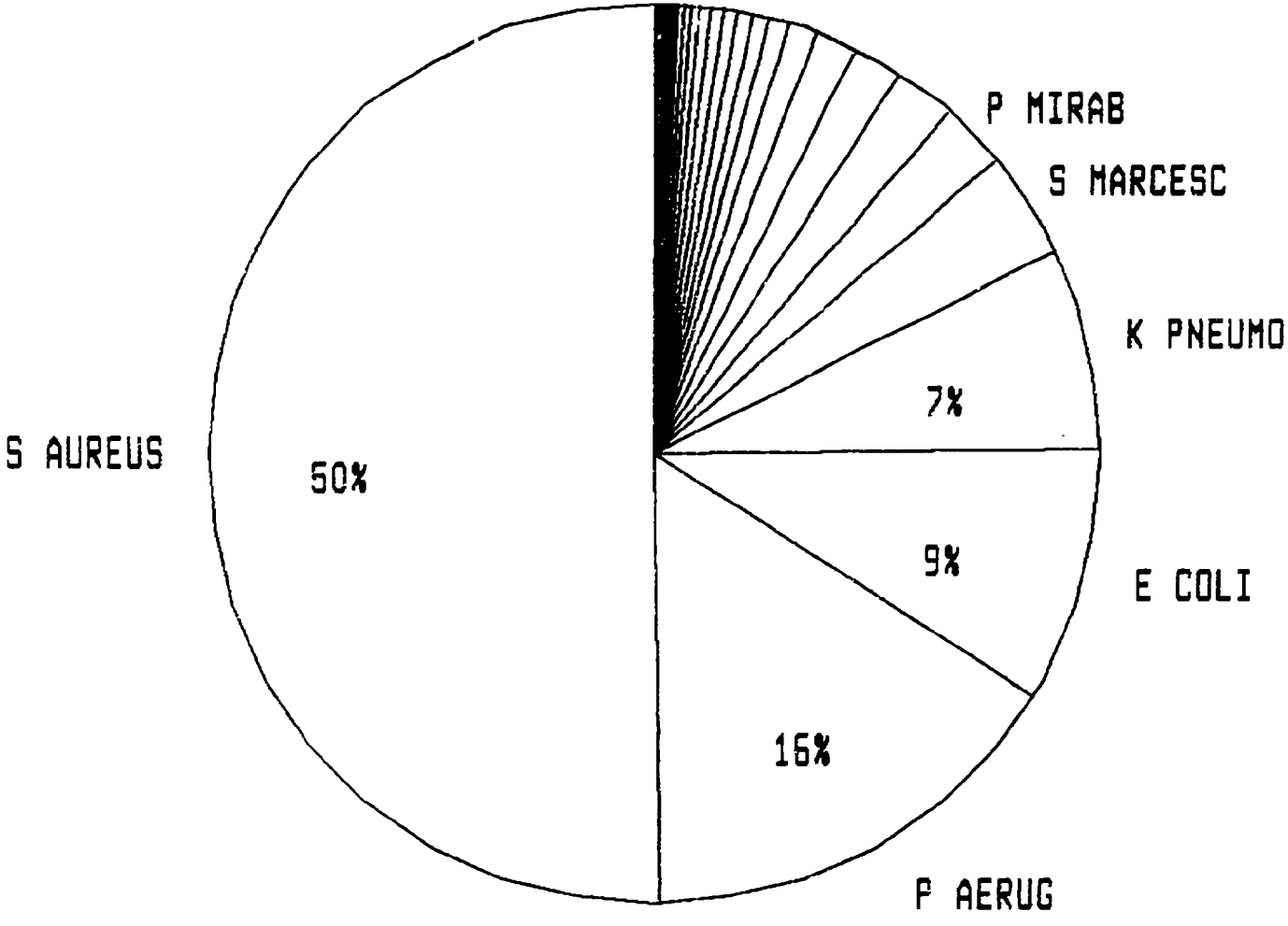


Figure 6. Display of the relative frequency of organisms tested for in vitro sensitivity to gentamicin in 1985.

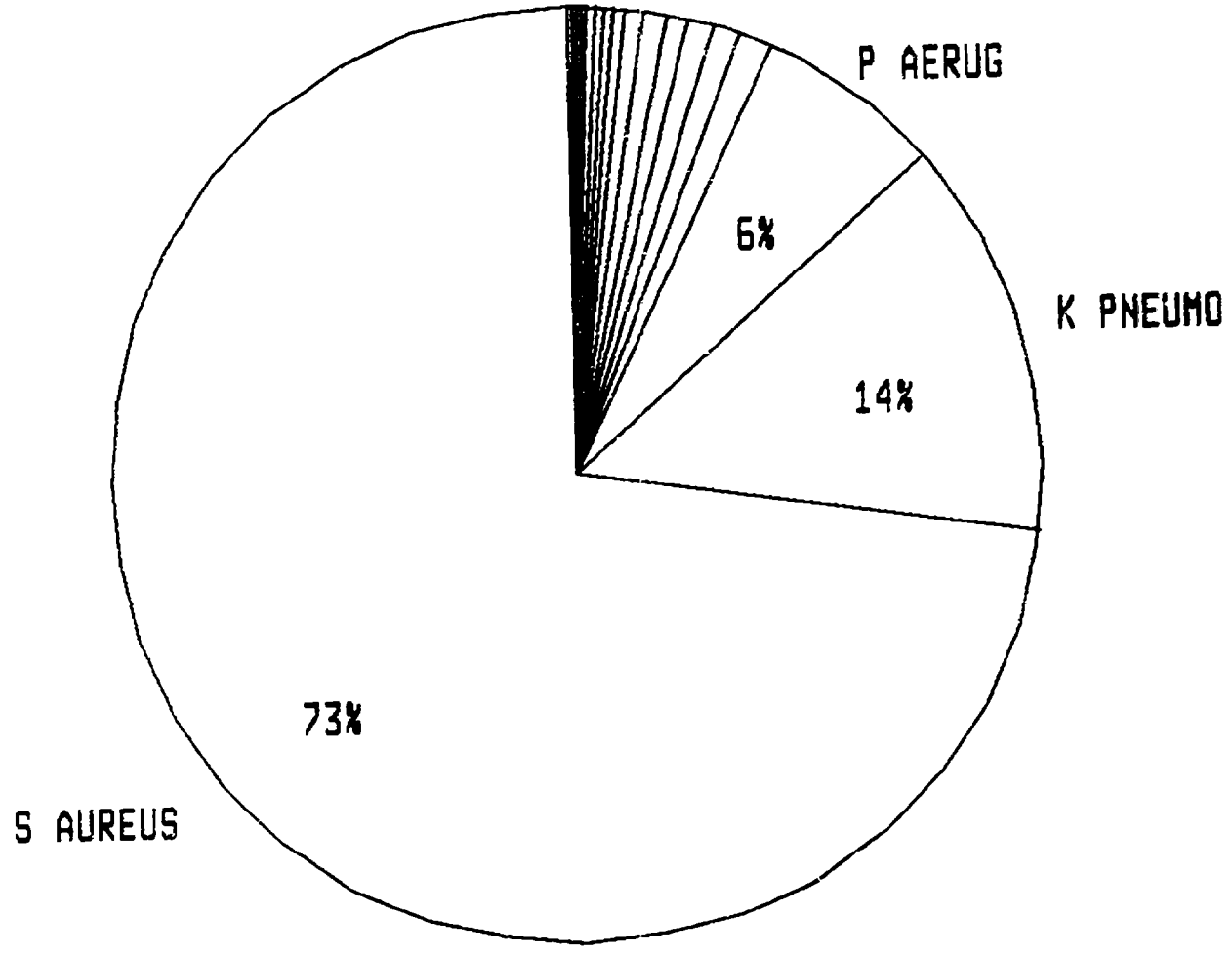


Figure 7. Display of the relative frequency of gentamicin-resistant organisms isolated in 1985.

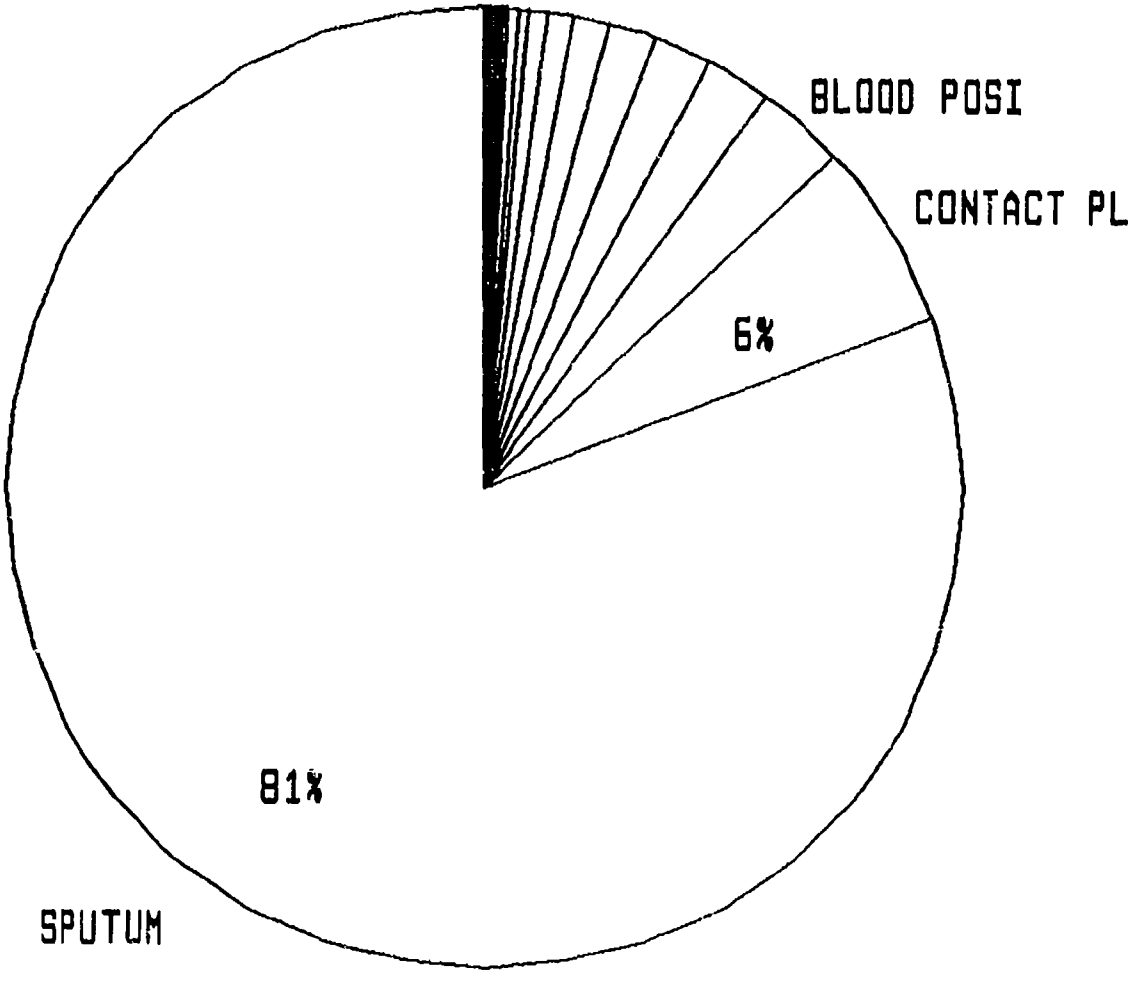


Figure 8. Display of the relative frequency of sources yielding Staphylococcus aureus tested for in vitro sensitivity to antibiotics in 1985.

TABLE 10. ANTIBIOTIC SENSITIVITY DATA FOR *Staphylococcus aureus* (1985)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	Percent	Number	Percent	Number	Percent	Number	Number
Amikacin	0.44	6	5.67	77	93.89	1,276	1,359
Ampicillin	29.43	400	7.73	105	62.84	854	1,359
Cefotaxime	0.37	5	24.45	332	75.18	1,021	1,358
Cefoperazone	0.07	1	28.84	392	71.08	966	1,359
Cefsulodin	0.37	5	0.00	0	99.63	1,350	1,355
Cephalothin	0.22	3	0.15	2	99.63	1,353	1,358
Chloramphenicol	0.22	3	0.88	12	98.90	1,344	1,359
Clindamycin	1.32	18	0.00	0	98.68	1,341	1,359
Erythromycin	29.87	406	6.03	82	64.09	871	1,359
Gentamicin	34.41	467	0.07	1	65.51	889	1,357
Kanamycin	35.61	484	0.22	3	64.16	872	1,359
Methicillin	10.66	144	25.82	276	63.52	679	1,069
Mezlocillin	95.66	1,300	0.22	3	4.12	56	1,359
MK0787	0.08	1	0.00	0	99.92	1,316	1,317
Moxalactam	20.90	284	17.00	231	62.10	844	1,359
Oxacillin	8.81	65	13.82	102	77.37	571	738
Penicillin	88.67	1,205	7.14	97	4.19	57	1,359
Piperacillin	95.58	1,297	0.07	1	4.35	59	1,357
Streptomycin	34.44	468	0.22	3	65.34	888	1,359
Sulfadiazine	36.72	499	22.15	301	41.14	559	1,359
Tetracycline	3.68	50	0.15	2	96.17	1,306	1,358
Tobramycin	34.76	472	0.07	1	65.17	885	1,358
Ticarcillin	94.56	1,285	0.00	0	5.44	74	1,359
Vancomycin	0.00	0	0.00	0	100.00	1,358	1,358

silver sulfadiazine, and chloramphenicol. Histogram displays of selected antibiotics are presented in Figure 9.

Pseudomonas aeruginosa. The frequency of sources of Pseudomonas aeruginosa strains tested in vitro is presented in Figure 10. The results of testing are presented in Table 11. Sensitivity of aminoglycoside antibiotics has remained low. The relative frequency of gentamicin resistance for recent reporting periods is presented in Figure 11. The relative frequency of sulfonamide resistance for recent reporting periods is presented in Figure 12. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 13.

Klebsiella pneumoniae. A total of 196 isolates were tested for in vitro sensitivities to antibiotics. The sources of isolation for tested strains are presented in Figure 14. The sensitivity results are presented in Table 12. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 15.

PRESENTATIONS/PUBLICATIONS

Mason AD Jr, McManus AT, and Pruitt BA Jr: Association of burn mortality and bacteremia: a twenty-five year review. Arch Surg 121:1027-1031, 1986.

Aulick LH, McManus AT, Pruitt BA Jr, and Mason AD Jr: Effects of oxygen consumption and core temperature in experimental thermal injury. Ann Surg 204:28-52, 1986.

Shirani KZ, McManus AT, Vaughan GM, McManus WF, Pruitt BA Jr, and Mason AD Jr: Effects of environment on infection in burn patients. Arch Surg 121:31-36, 1986.

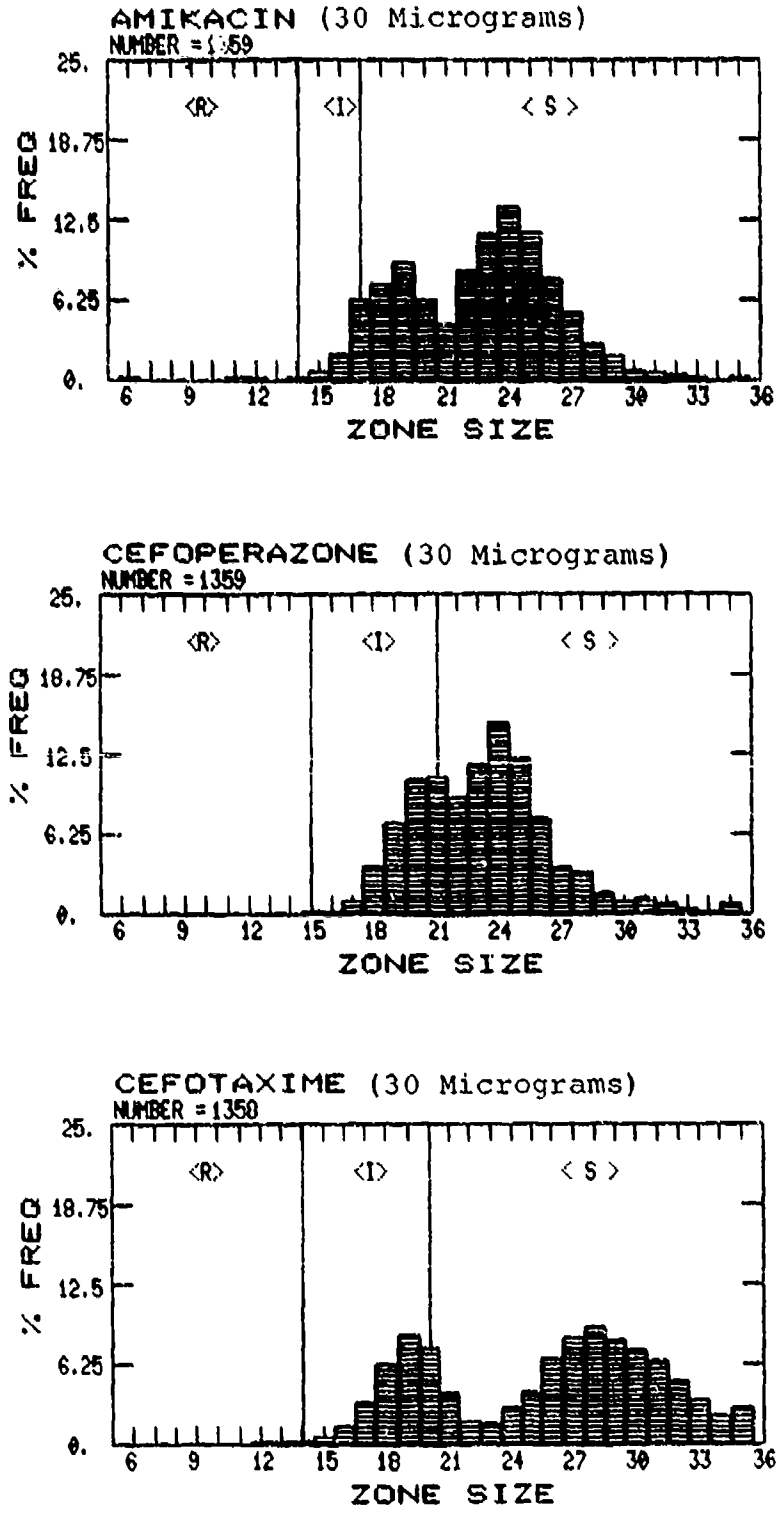


Figure 9. Histogram display of the distribution of zones of inhibition of growth of Staphylococcus aureus.

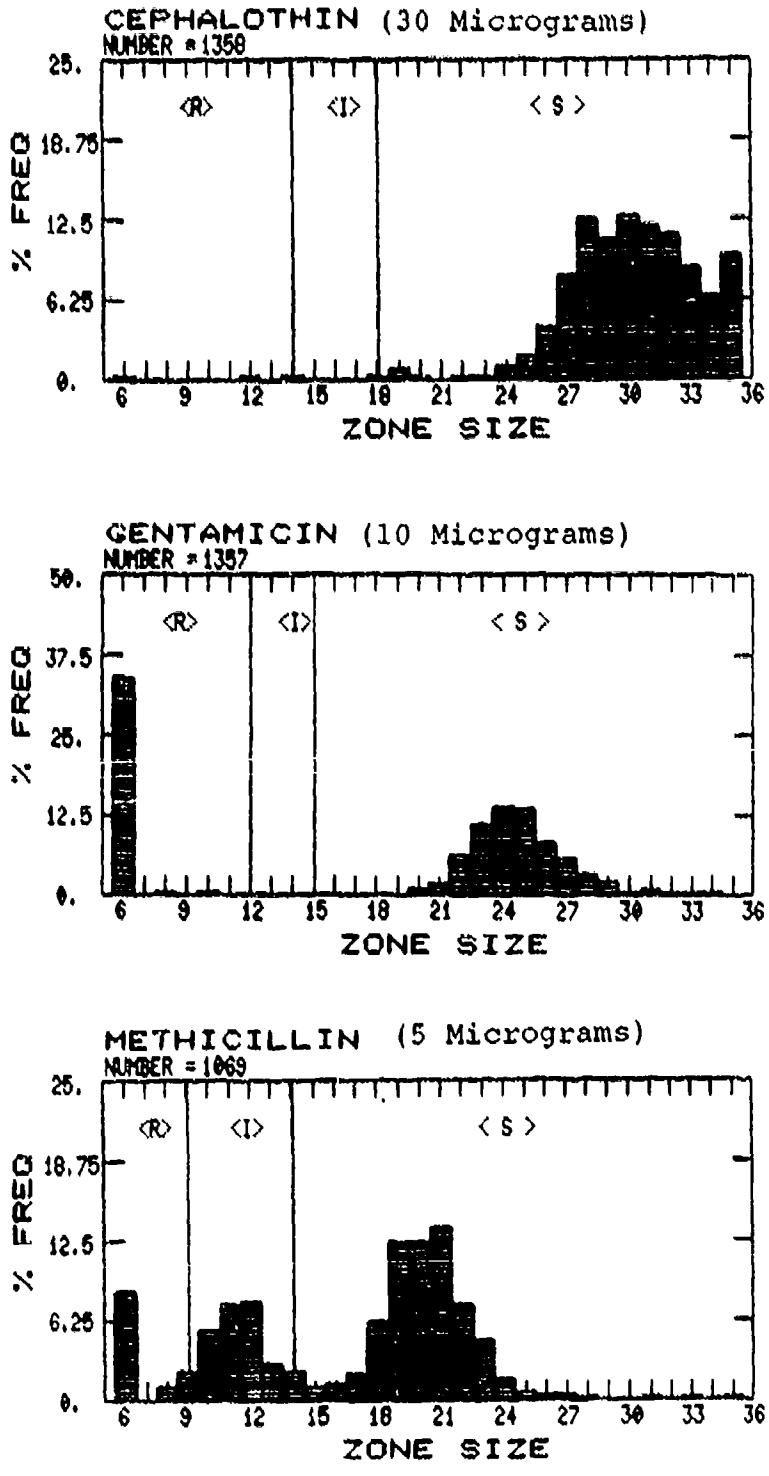


Figure 9. Histogram display of the distribution of zones of inhibition of growth of Staphylococcus aureus (continued).

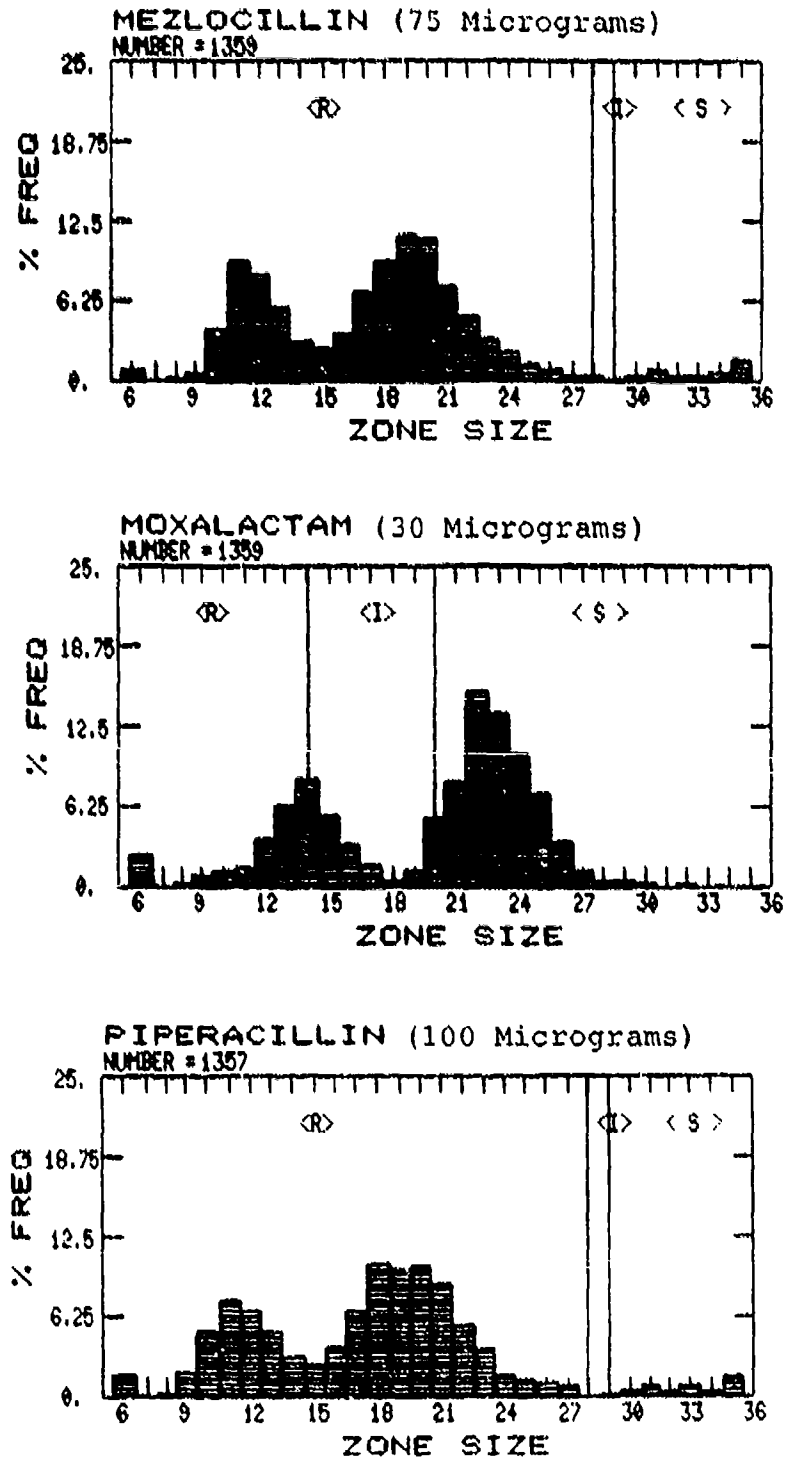


Figure 9. Histogram display of the distribution of zones of inhibition of growth of Staphylococcus aureus (continued).

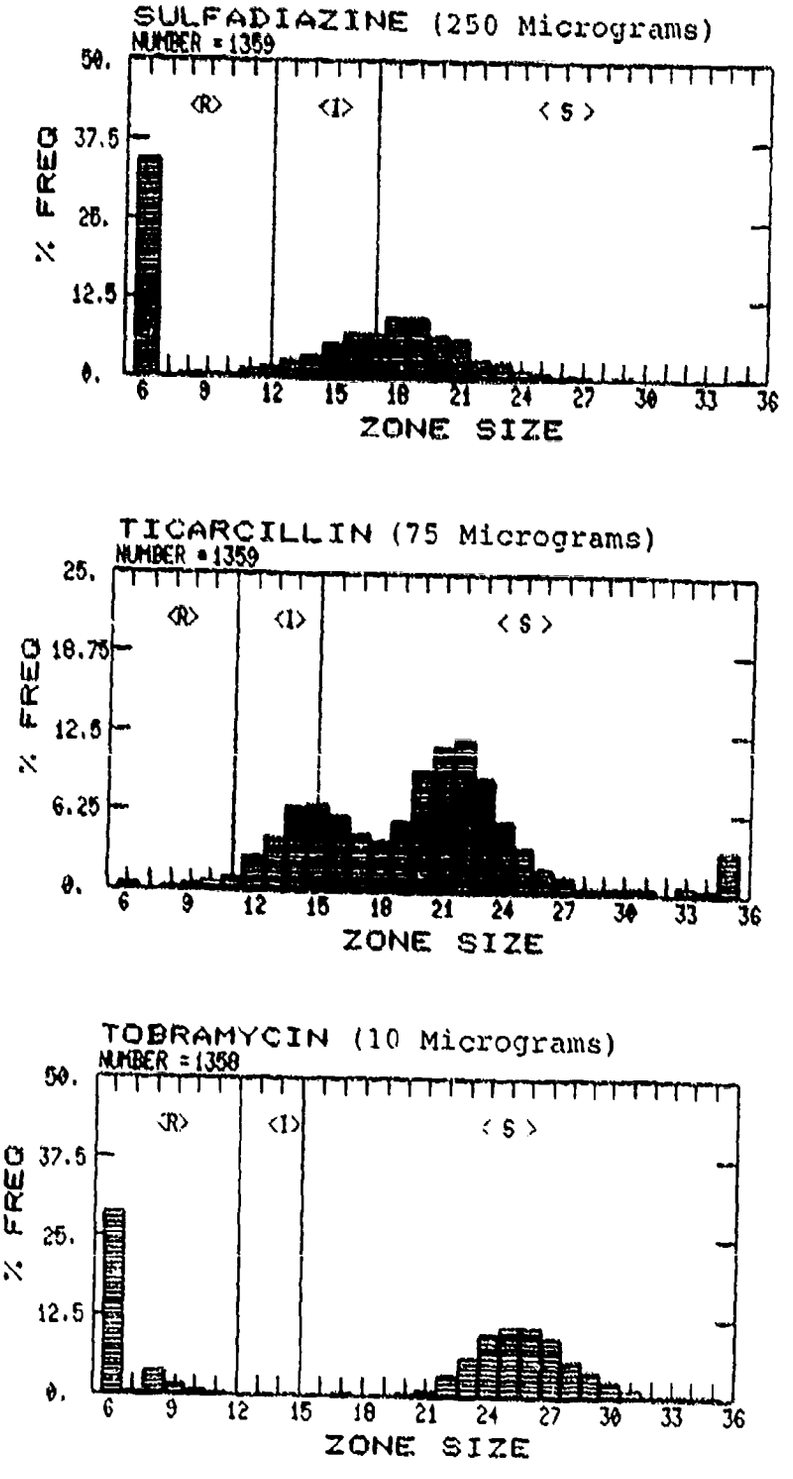


Figure 9. Histogram display of the distribution of zones of inhibition of growth of Staphylococcus aureus (continued).

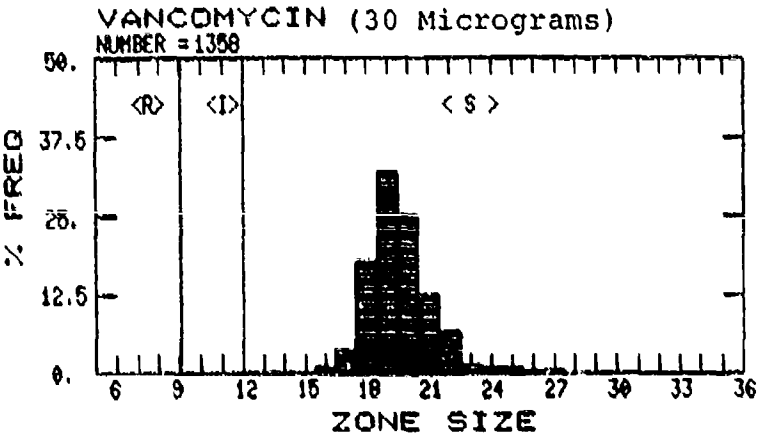


Figure 9. Histogram display of the distribution of zones of inhibition of growth of Staphylococcus aureus (continued).

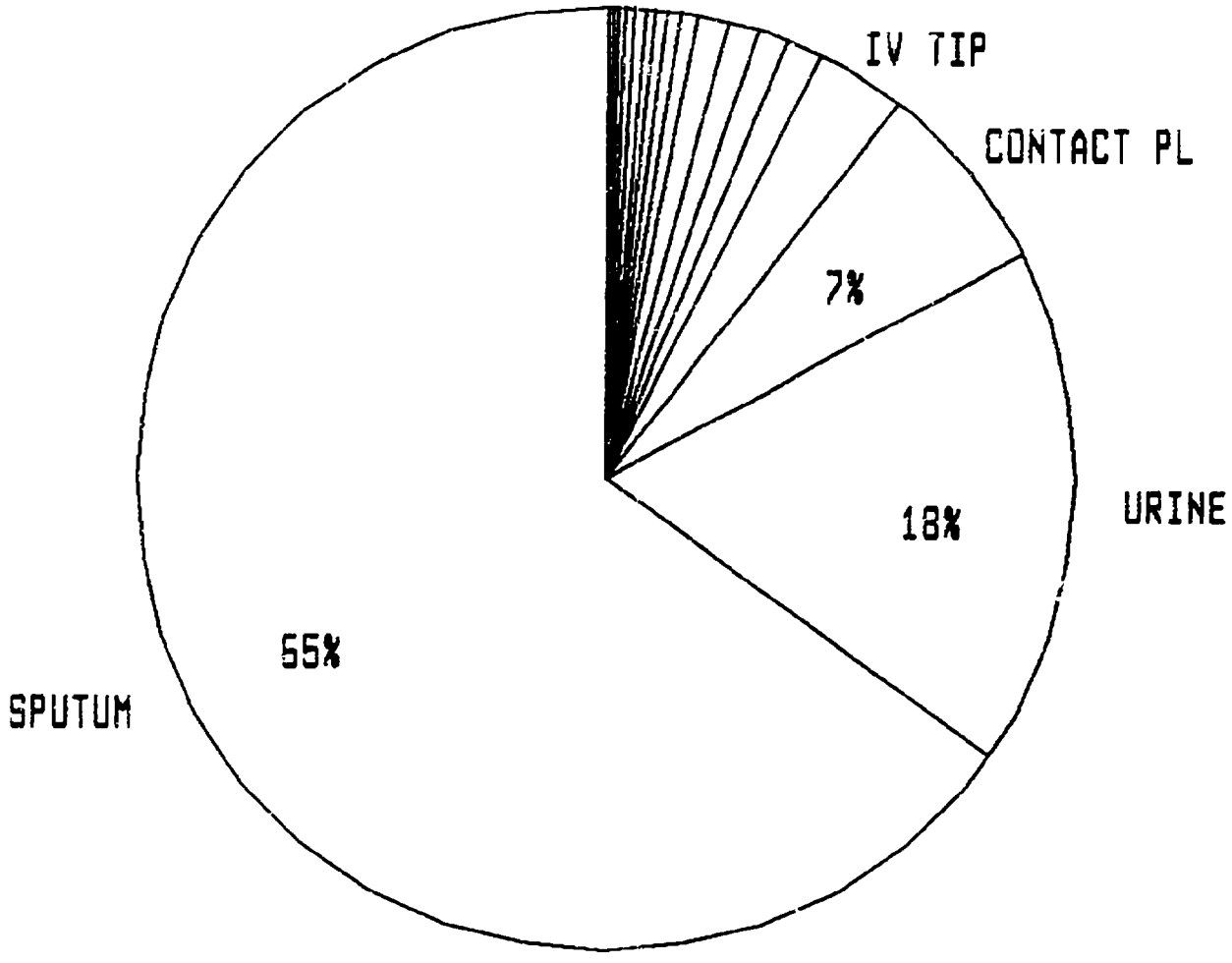


Figure 10. Display of the relative frequency of sources yielding Pseudomonas aeruginosa tested for in vitro sensitivity to antibiotics in 1985.

TABLE 11. ANTIBIOTIC SENSITIVITY DATA FOR *Pseudomonas aeruginosa* (1985)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	Percent	Number	Percent	Number	Percent	Number	Number
Amikacin	7.24	31	18.22	78	74.53	319	428
Azlocillin	28.40	121	4.69	20	66.90	285	426
Aztreonam	10.30	41	29.15	116	60.55	241	398
Cefoperazone	15.49	66	19.72	84	64.79	276	426
Cefotaxime	40.65	174	49.30	211	10.05	43	428
Cefsulodin	11.75	49	3.12	13	85.13	355	417
Chloramphenicol	85.98	368	13.32	57	0.70	3	428
Colistin	0.00	0	1.87	8	98.13	420	428
Gentamicin	9.58	41	31.31	134	59.11	253	428
Kanamycin	97.66	418	1.87	8	0.47	2	428
Mezlocillin	34.58	148	13.08	56	52.34	224	428
MK0787	9.91	42	6.13	26	83.96	356	424
Moxalactam	34.81	149	48.60	208	16.59	71	428
Netilmicin	25.93	111	3.50	15	70.56	302	428
Norfloxacin	0.00	0	1.76	7	98.24	391	398
Piperacillin	14.02	60	13.79	59	72.20	309	428
Sulfadiazine	17.61	75	19.01	81	63.38	270	426
Tetracycline	90.19	386	7.24	31	2.57	11	428
Tobramycin	24.07	103	2.57	11	73.36	314	428
Ticarcillin	13.08	56	15.65	67	71.26	305	428
TIM-85	28.13	92	4.28	14	67.58	221	327

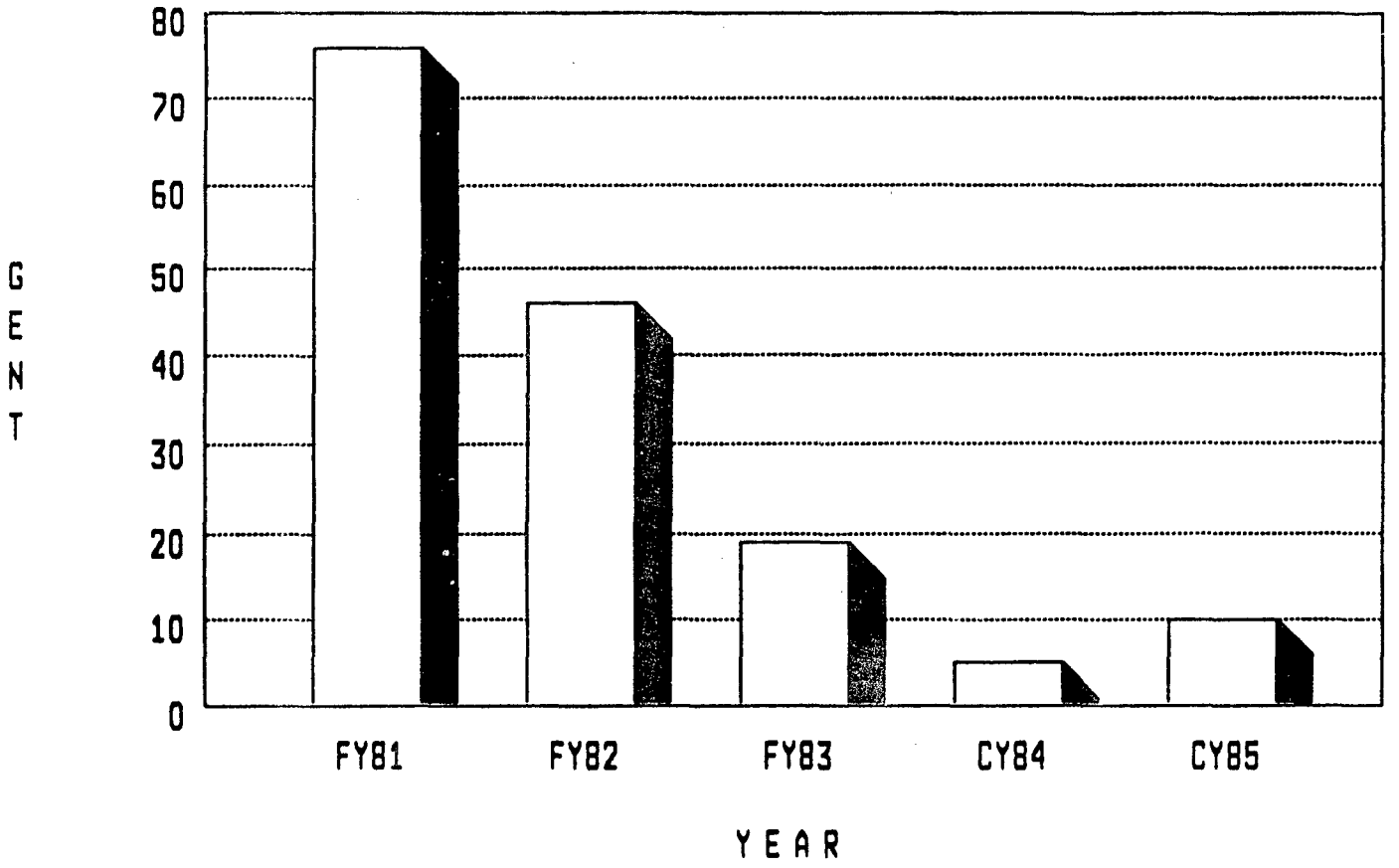


Figure 11. Relative frequency of Pseudomonas aeruginosa resistant to gentamicin (percent) for fiscal years 1981 through 1983 and calendar years 1984 and 1985.

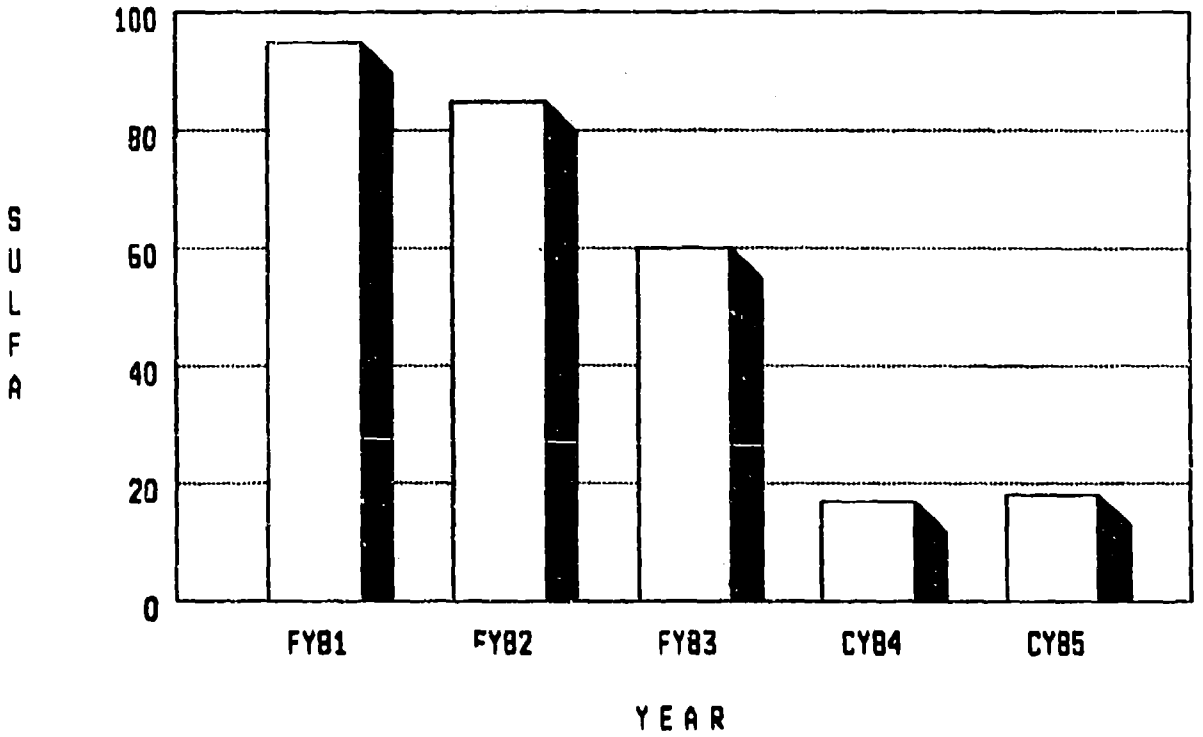


Figure 12. Relative frequency of Pseudomonas aeruginosa resistance to sulfonamides (percent) for fiscal years 1981 through 1983 and calendar years 1984 and 1985.

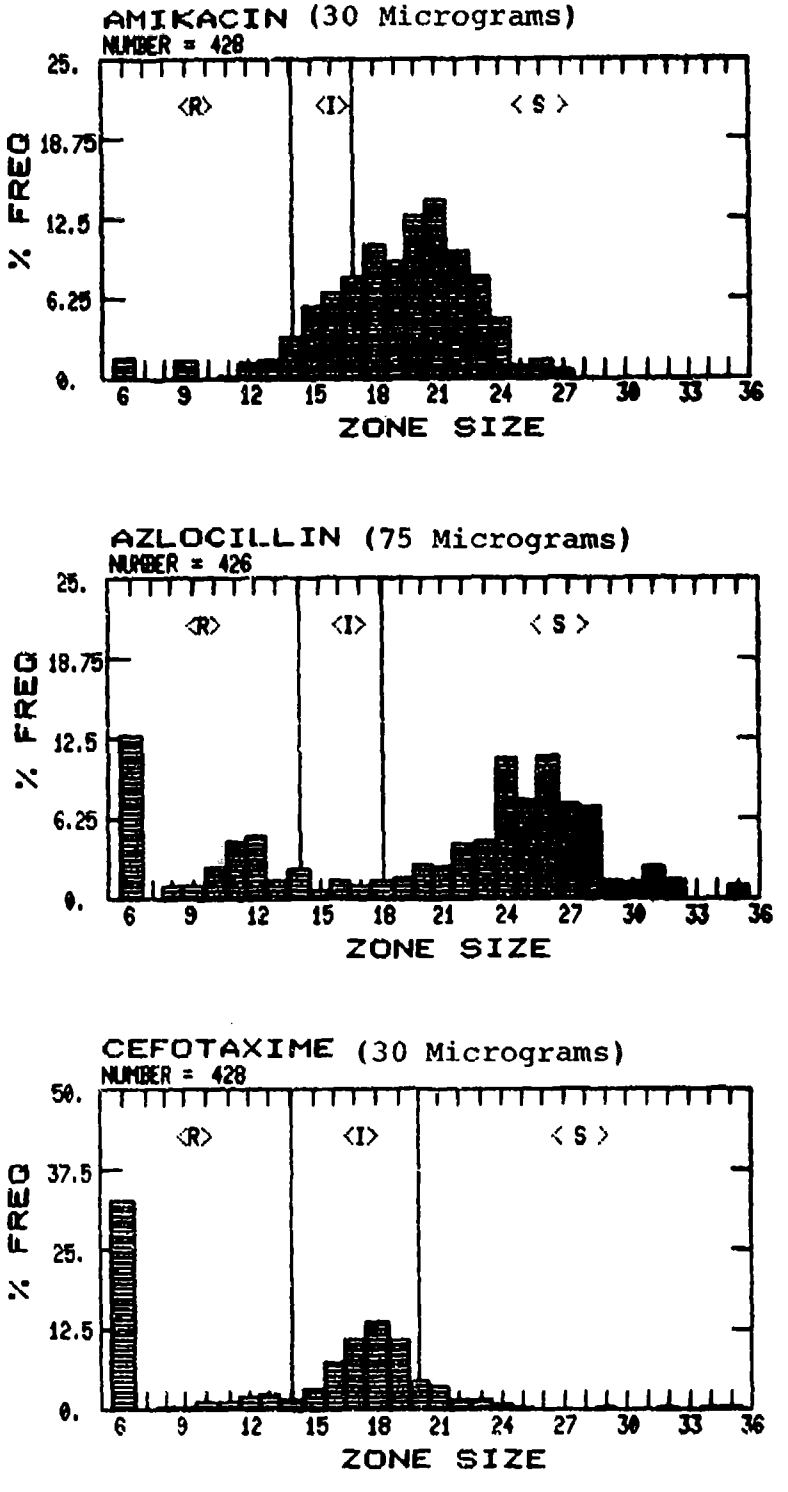


Figure 13. Histogram display of the distribution of zones of inhibition of growth of Pseudomonas aeruginosa.

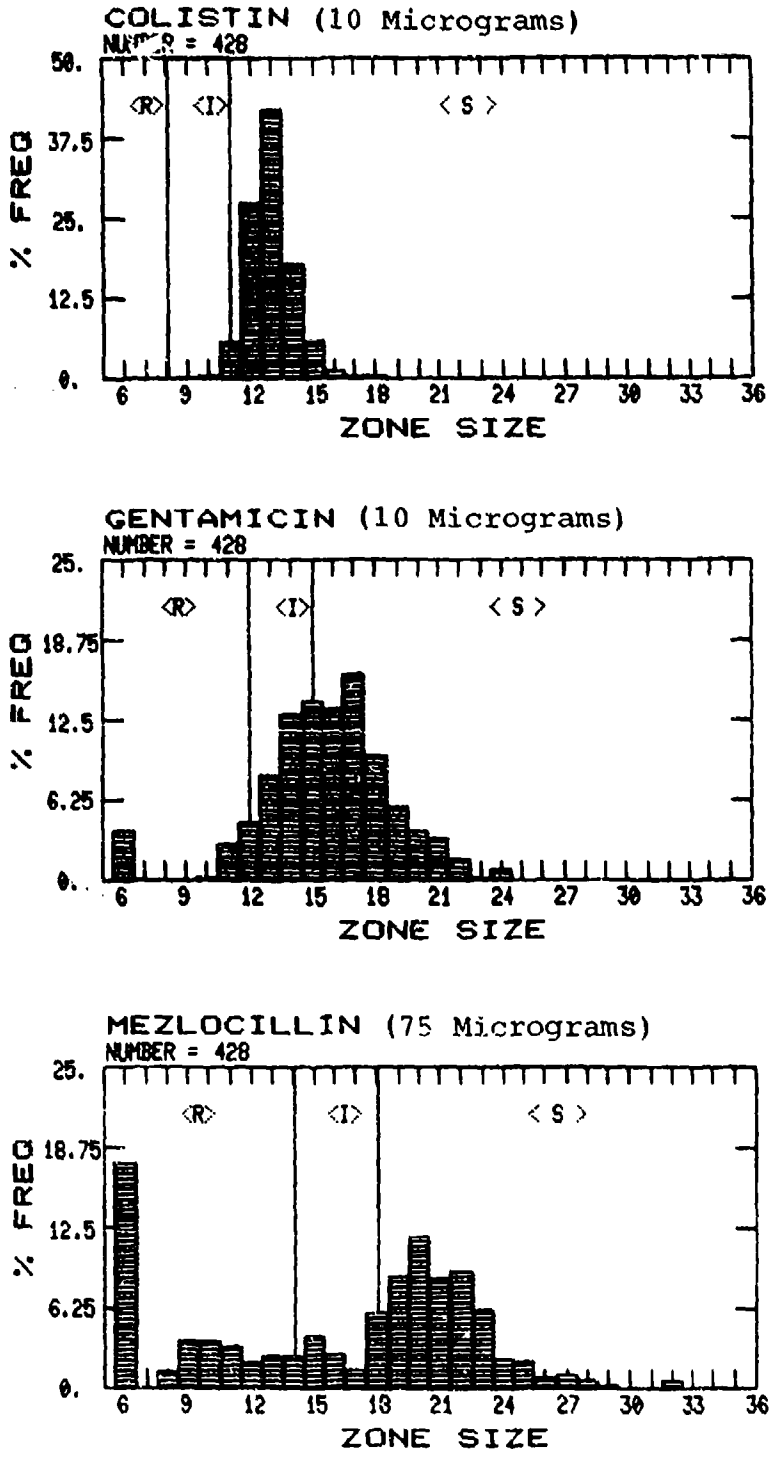


Figure 13. Histogram display of the distribution of zones of inhibition of growth of Pseudomonas aeruginosa (continued).

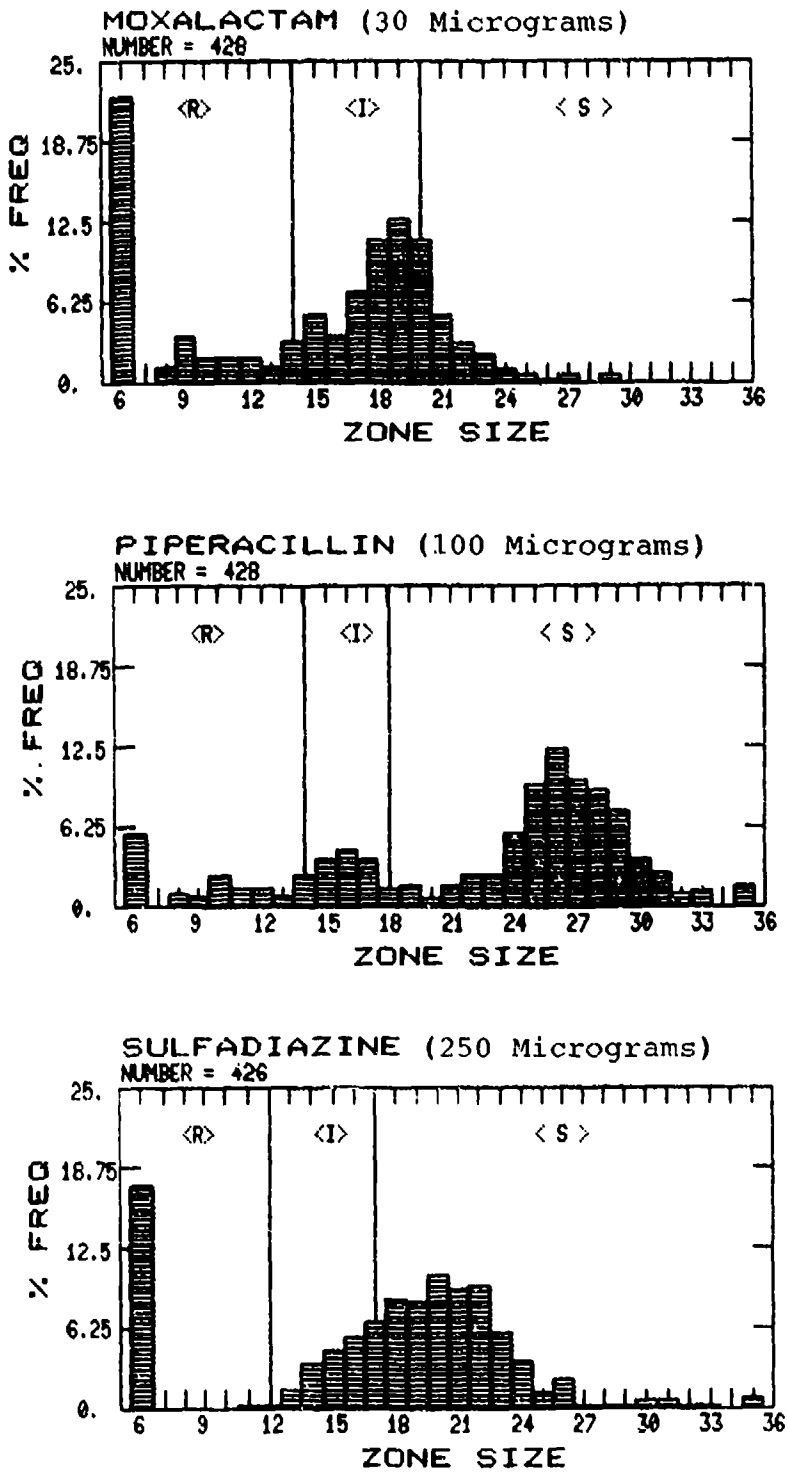
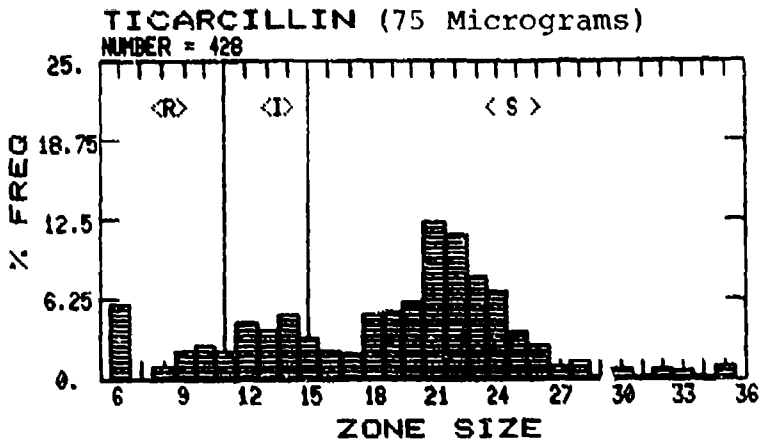


Figure 13. Histogram display of the distribution of zones of inhibition of growth of Pseudomonas aeruginosa (continued).



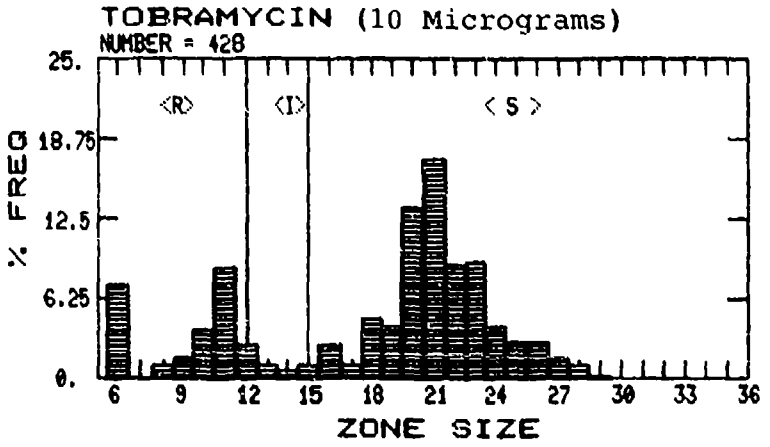


Figure 13. Histogram display of the distribution of zones of inhibition of growth of Pseudomonas aeruginosa (continued).

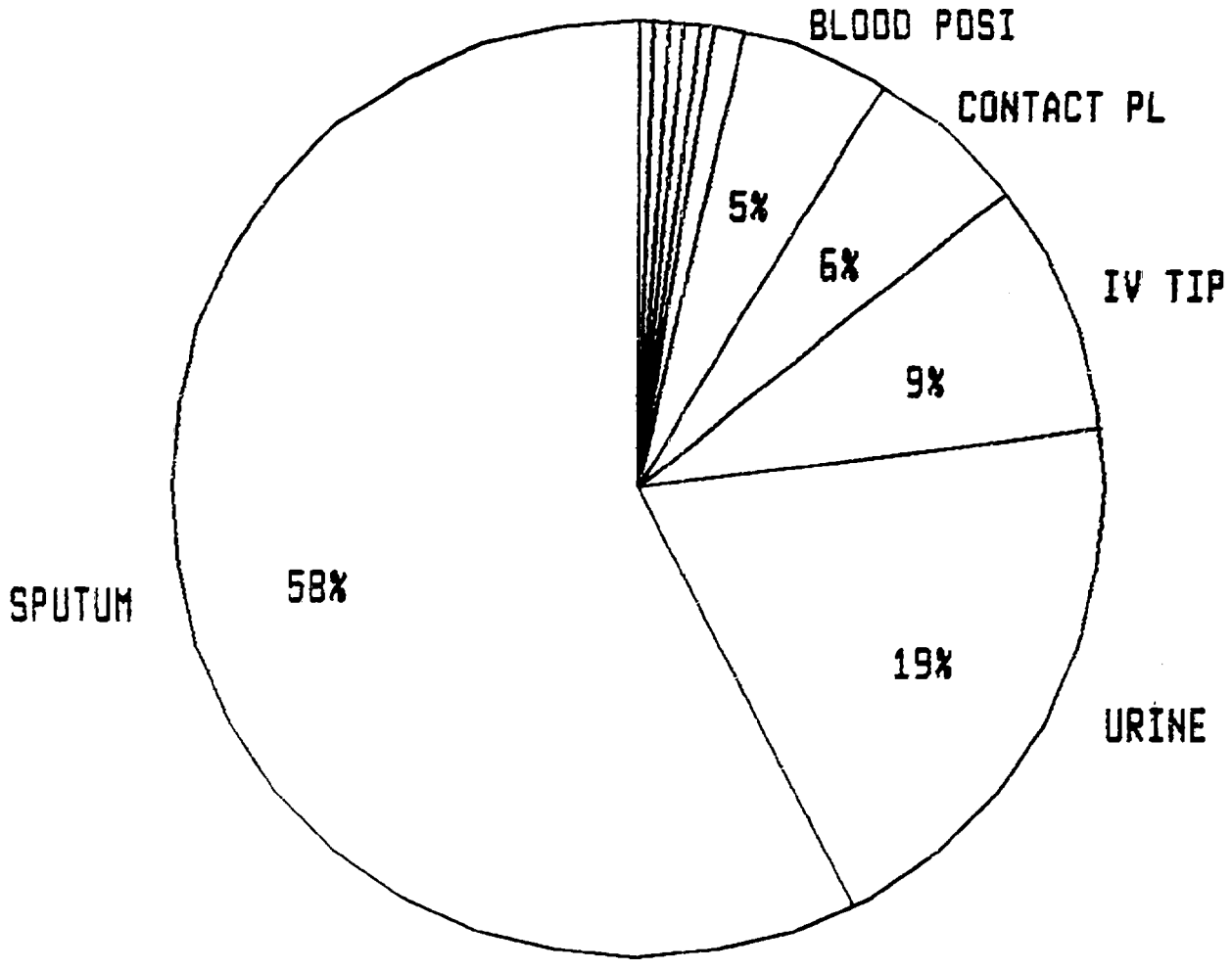


Figure 14. Display of the relative frequency of sources yielding Klebsiella pneumoniae tested for in vitro sensitivity to antibiotics in 1985.

TABLE 12. ANTIBIOTIC SENSITIVITY DATA FOR Klebsiella pneumoniae (1985)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	Percent	Number	Percent	Number	Percent	Number	Number
Amikacin	0.00	0	27.04	53	72.96	143	196
Ampicillin	65.46	127	18.56	36	15.98	31	194
Aztreonam	0.00	0	0.00	0	100.00	181	181
Cefamandole	19.07	37	26.80	52	54.12	105	194
Cefoperazone	0.51	1	44.90	88	54.59	107	196
Cefotaxime	0.00	9	0.00	0	100.00	196	196
Cefoxitin	0.52	1	3.09	6	96.39	187	196
Chloramphenicol	46.94	92	0.00	0	53.06	104	196
Gentamicin	44.90	88	0.00	0	55.10	108	196
Kanamycin	45.41	89	3.57	7	51.02	100	196
Mezlocillin	46.94	92	7.14	14	45.92	90	196
MK0787	0.00	0	0.00	0	100.00	187	187
Moxalactam	0.00	0	0.51	1	99.49	195	196
Nalidixic Acid	0.51	1	0.51	1	98.98	194	196
Neomycin	0.00	0	0.00	0	100.00	14	14
Netilimicin	39.69	77	4.64	9	55.67	108	194
Norfloxacin	0.00	0	0.00	0	100.00	180	180
Piperacillin	45.92	90	2.04	4	52.04	102	196
Sulfadiazine	59.69	117	5.10	10	35.20	69	196
Streptomycin	2.04	4	11.22	22	86.73	170	196
Tetracycline	6.12	12	8.16	16	85.71	168	196
Tobramycin	44.90	88	0.51	1	54.49	107	196
Ticarcillin	79.59	156	7.14	14	13.27	26	196
Trimethoprim	0.51	1	0.00	0	99.49	195	196
Trimeth & Sulfa	0.51	1	2.04	4	97.45	191	196

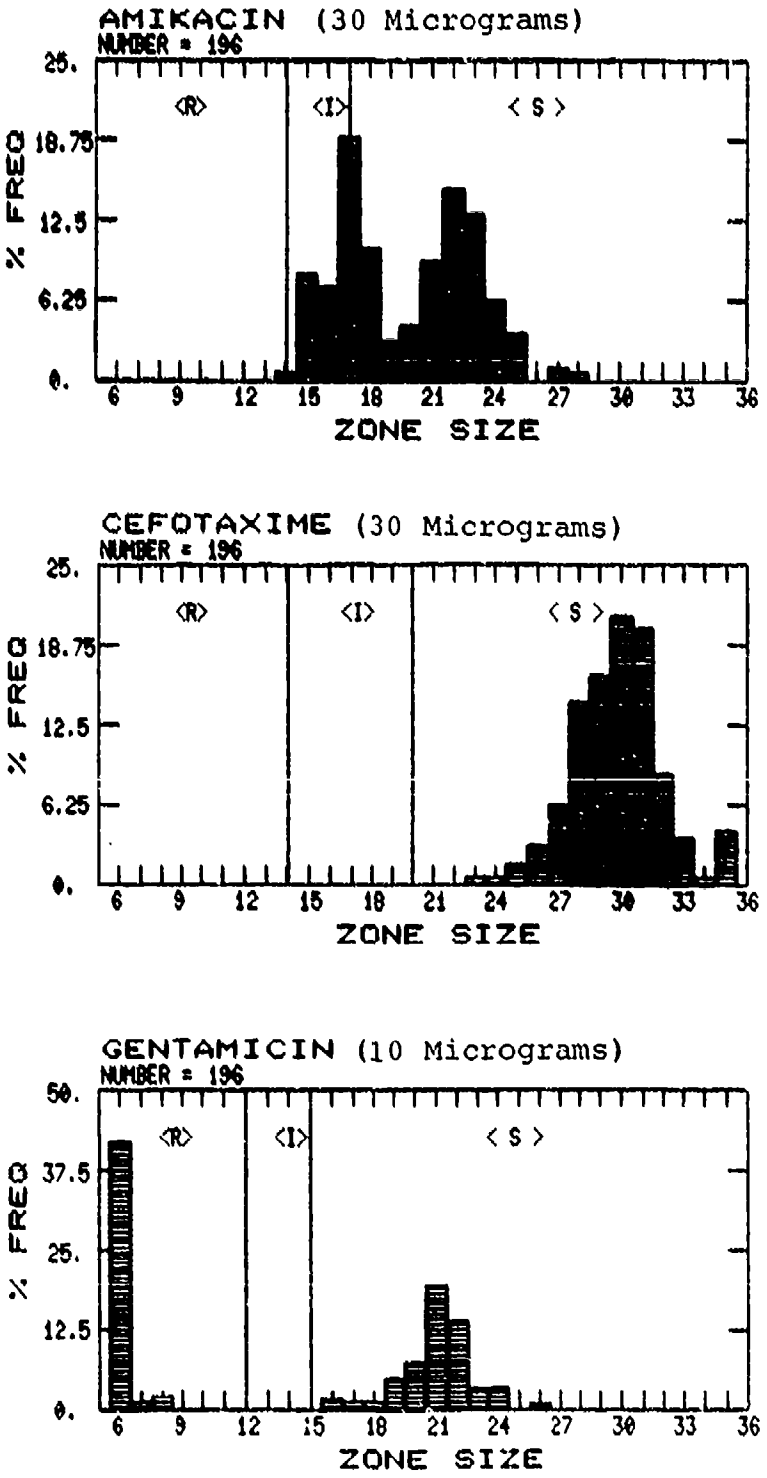


Figure 15. Histogram display of the distribution of zones of inhibition of growth of Klebsiella pneumoniae.

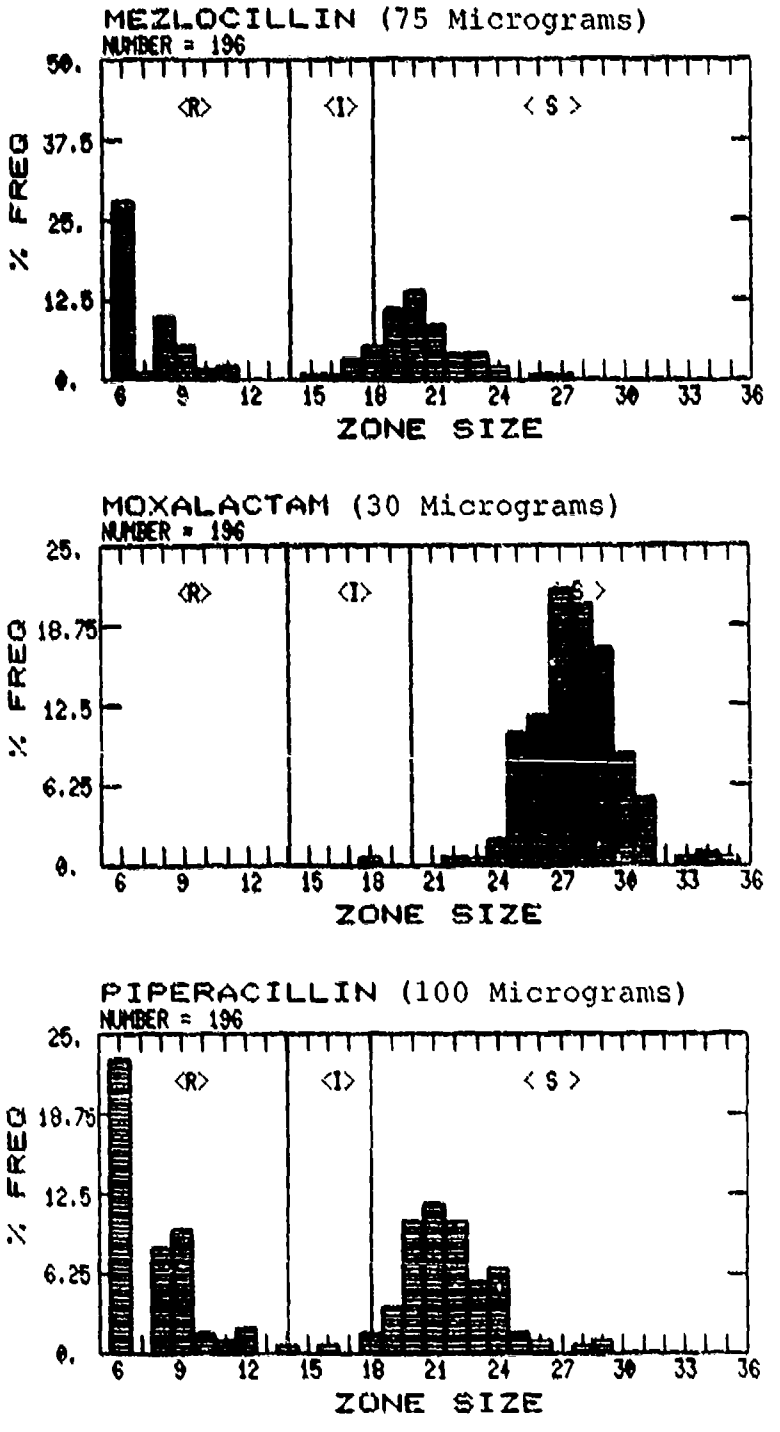


Figure 15. Histogram display of the distribution of zones of inhibition of growth of Klebsiella pneumoniae (continued).

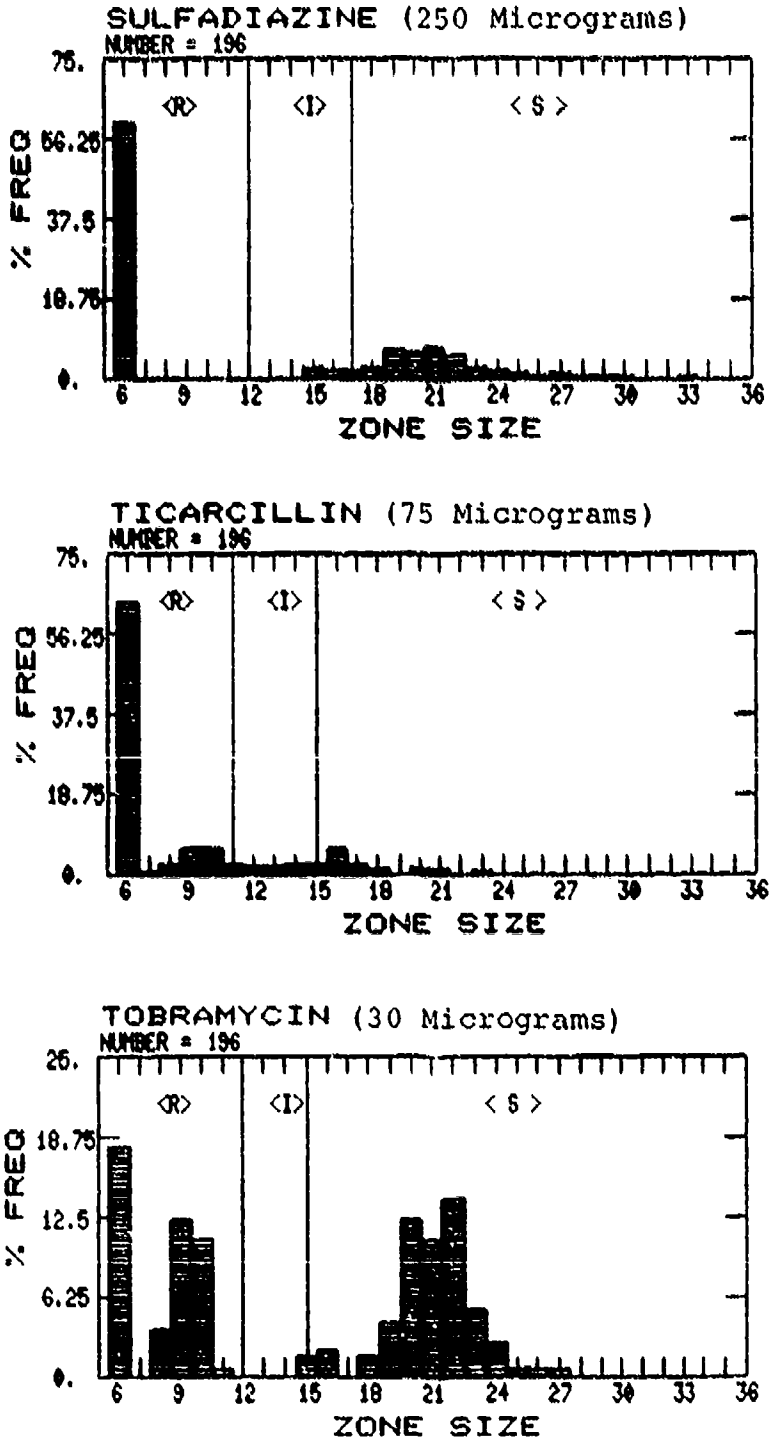


Figure 15. Histogram display of the distribution of zones of inhibition of growth of Klebsiella pneumoniae (continued).

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: Effectiveness of Mafenide Acetate and Silver Sulfadiazine in the Scalded Mouse Model

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

George B. Stover, MS, Captain, MS
Albert T. McManus, PhD
Gene B. Hubbard, DVM, MS, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: Effectiveness of Mafenide Acetate and Silver Sulfadiazine in the Scalded Mouse Model

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: George B. Stover, MS, Captain, MS
Albert T. McManus, PhD
Gene B. Hubbard, DVM, MS, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

To validate the scalded mouse as a model for testing topical chemotherapeutic agents for burn treatment, two such agents, mafenide acetate and silver sulfadiazine were tested against Pseudomonas aeruginosa, Strains 59-1244 and VA-134, and Proteus mirabilis, Strain 77082234. Anesthetized mice received 12-square centimeter full-thickness dorsal burns by exposure to 80° C water for six seconds using a watertight template. Groups of 10 mice were inoculated within one hour of scalding with the test organism. Topical chemotherapy was begun at four or 24 hours postburn and continued once per day for 10 days. Controls included inoculated/untreated and burn-only groups. No deaths occurred in the burn-only group. With treatment started four hours postburn, the scalded mouse results were qualitatively and quantitatively similar to published results using the standard scalded rat model.

EFFECTIVENESS OF MAFENIDE ACETATE AND SILVER SULFADIAZINE IN THE SCALDED MOUSE MODEL

INTRODUCTION

The use and continued development of topical chemotherapy has greatly reduced the occurrence of invasive bacterial burn wound infections (1-2). Invasive burn wound sepsis caused by Pseudomonas aeruginosa and Proteus mirabilis has been previously demonstrated in the scalded mouse model (3). In the present study, we have utilized the scalded mouse model to investigate the relative efficacy of mafenide acetate and sulfadiazine silver against infection by Pseudomonas aeruginosa or Proteus mirabilis (4-6).

MATERIALS AND METHODS

Bacteria. Pseudomonas aeruginosa, Strains 59-1244 (7) and VA-134, and Proteus mirabilis, Strain 77082234 (8), were used throughout this study.

Quantitation of Bacteria. Bacteria were grown in brain-heart infusion broth for 16 hours at 37° C on a gyratory shaker. Cells were harvested by centrifugation (4,000 times gravity for 15 minutes), washed, and diluted in a standard

---

[1]Pruitt BA Jr and McManus AT: Opportunistic infections in severely burned patients. Am J Med 76:146-154, 1984.

[2]Pruitt BA Jr and Lindberg RB: Pseudomonas aeruginosa infections in burn patients. Doggett RG (ed). In Pseudomonas aeruginosa: clinical manisfestations of infection and current therapy. New York: Academic Press, pp 339-366.

[3]Stover GB, Hubbard GB, Mason AD Jr, and McManus AT: (Abstract). Proceedings of the 86th Annual Meeting of the American Society for Microbiology B112:43, 1986.

[4]Lindberg RB, Moncrief JA, Switzer WE, et al: The successful control of burn wound sepsis. J Trauma 5:601-616, 1965.

[5]Moncrief JA, Lindberg RB, Switzer WE, et al: The use of a topical sulfonamide in the control of burn wound sepsis. J Trauma 6:407-419, 1966.

[6]Lindberg RB, Moncrief JA, and Mason AD Jr: Control of experimental and clinical burn wound sepsis by topical application of sulfamylon compounds. Ann NY Acad Sci 150:950-960, 1968.

[7]Walker HL, Mason AD Jr, and Raulson GL: Surface infection with Pseudomonas aeruginosa. Ann Surg 160:297-305, 1964.

[8]McManus AT, McLeod CG, and Mason AD Jr: Experimental Proteus mirabilis burn surface infection. Arch Surg 117:187-191, 1982.

diluent (25 millimeters potassium phosphate, pH 7.0, 0.9 percent sodium chloride, 0.01 percent gelatin, 0.2 millimeters magnesium sulfate-$_7H_2O$). Viable cell counts were determined by spiral plating (Spiral Systems, Bethesda, Maryland) on trypticase soy agar plates. Plates were counted after 24 hours incubation at 37° C by a laser bacteria colony counter (Model 500A, Spiral Systems).

Laboratory Animals. Male Harlan Sprague-Dawley mice weighing approximately 26 grams were used in all experiments. Prior to experimental use, the animals were maintained in wire-bottomed cages at 30 mice per cage with commercial rodent chow and water available ad libitum.

Experimental Burn Procedure. Experimental and control animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (0.5 milliliters of a 1:20 dilution). The backs of mice were then shaved with an Oster small animal clipper (blade size = 40). Each mouse was placed in a fixed area shield (12 square centimeters) and the dorsum was immersed in 80° C water for seven seconds. This resulted in a third degree burn of approximately 15 percent of the total body surface area. Following the scald, all mice were injected intraperitoneally with 0.5 milliliters of sterile physiological saline. Animals were challenged immediately postburn by topical application of 0.3 milliliters of a bacterial suspension (100-1,000 LD_{50}). Control mice were scalded but not inoculated.

Topical Treatment. Topical chemotherapy was attempted with 11-percent mafenide acetate (Sulfamylon® Cream, Winthrop-Breon laboratories, 90 Park Avenue, New York, New York 10016) and one-percent sulfadiazine silver (Silvadene® Cream, Marion Laboratories, Inc., Kansas City, Missouri 64137). Treatment was initiated at either four or 24 hours postburn and was continued daily for a total of 10 applications.

Histologic Examination. Complete sets of tissue were fixed in 10-percent neutral buffered formalin and processed by standard methods.

RESULTS

The efficacy of mafenide acetate and silver sulfadiazine in the scalded rat model has been previously reported (9-10). Table 1 shows the survival rates of mice infected with Pseudomonas aeruginosa Strain 59-1244 and treated beginning four hours postburn. Both agents were effective. The results using a second invasive strain of Pseudomonas aeruginosa, Strain VA-134, are shown in Table 2. These results were similar to those shown for Strain 59-1244. Mafenide acetate was found to be ineffective against infection with Proteus

TABLE 1

MORTALITY (NUMBER OF DEAD VERSUS NUMBER IN GROUP) IN BURNED, *Pseudomonas aeruginosa*-INFECTED (STRAIN 59-1244) MICE

Challenge Dose	DAY POSTBURN							Cumulative Mortality
	1	2	3	4	5	6	7*	
Burn Control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Untreated	0/10	8/10	10/10	-	-	-	-	10/10
Sulfamylon Treatment**	0/10	1/10	2/10	2/10	2/10	2/10	2/10†	2/10
Silver Sulfadiazine Treatment**	0/10	0/10	0/10	0/10	0/10	0/10	0/10†	0/10

*No further deaths for 21 days postburn.
**Treatment begun at 4 hours postburn.
†Not significant (Fisher's Exact Test).

TABLE 2

MORTALITY (NUMBER OF DEAD VERSUS NUMBER IN GROUP) IN BURNED, *Pseudomonas aeruginosa*-INFECTED (STRAIN VA-134) MICE

Challenge Dose	DAY POSTBURN							Cumulative Mortality
	1	2	3	4	5	6	7*	
Burn Control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Untreated	0/10	7/10	10/10	-	-	-	-	10/10
Sulfamylon Treatment**	0/10	2/10	2/10	2/10	2/10	2/10	2/10†	2/10
Silver Sulfadiazine Treatment**	0/10	1/10	1/10	1/10	1/10	1/10	1/10†	1/10

*No further deaths for 21 days postburn.
**Treatment begun at 4 hours postburn.
†Not significant (Fisher's Exact Test).

mirabilis (Table 3). However, treatment with silver sulfadiazine resulted in 100-percent survival.

The time of initiation of therapy has been reported to be critical in the prevention of burn wound sepsis (6). The present study used a second series of experiments that involved beginning antibiotic treatment at 24 hours postburn. Mortality rates worsened in mice infected with either of the two strains of Pseudomonas aeruginosa. Table 4 shows the survival rates of mice infected with Pseudomonas aeruginosa Strain 59-1244. All infected, untreated mice died along with 80 percent of the mice treated with mafenide acetate and 40 percent of the mice treated with silver sulfadiazine. When treatment was delayed until 24 hours, Strain VA-134 showed little therapeutic activity for either agent (Table 5). When treatment was delayed for 24 hours, there was a 100-percent mortality in all groups of mice infected with Proteus mirabilis (Table 6).

Histologic examination of mice treated beginning 24 hours postburn showed results similar to previous reports of untreated infected animals (11-12). Hepatic necrosis was widespread among untreated and treated animals. The findings in mice treated beginning four hours postburn were different. Hepatic necrosis in untreated Pseudomonas aeruginosa-infected mice was again common. However, mafenide acetate or silver sulfadiazine-treated mice showed no evidence of hepatic necrosis. Hepatic necrosis in untreated and mafenide acetate-treated Proteus mirabilis-infected mice was common (Figure 1). Proteus mirabilis-infected mice treated with

---

[9]Lindberg RB, Moncrief JA, Brame RE, et al: A comparison of sulfamylon hydrochloride and sulfamylon acetate in control of experimental burn wound infections. In US Army Surgical Research Unit Annual Research Progress Report. San Antonio: US Army Surgical Research Unit, 1966, pp 40 - 40-6.

[10]Lindberg RB, Brame RE, Mason AD Jr, et al: Development of prophylactic topical therapy for use on burn wounds: search for improved formulations. In US Army Institute of Surgical Research Annual Research Progress Report. San Antonio: US Army Institute of Surgical Research, 1971, pp 7 - 7-17.

[11]Stover GB, Hubbard GB, Mason AD Jr, et al: Comparison of virulence of Pseudomonas aeruginosa in burned rats and mice. In US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1984. San Antonio: US Army Institute of Surgical Research, pp 184-195.

[12]Stover GB, Hubbard GB, Mason AD Jr, et al: Comparison of scalded rat-standardized strains of Pseudomonas aeruginosa and Proteus mirabilis in the scalded mouse. In US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1985. San Antonio: US Army Institute of Surgical Research, pp 227-238.

TABLE 3

MORTALITY (NUMBER OF DEAD VERSUS NUMBER IN GROUP) IN BURNED, *Proteus mirabilis*-INFECTED (STRAIN 77082234) MICE

Challenge Dose	DAY POSTBURN 1	2	3	4	5	6	7*	Cumulative Mortality
Burn Control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Untreated	4/10	10/10	-	-	-	-	-	10/10
Sulfamylon Treatment**	2/10	9/10	10/10†	-	-	-	-	10/10
Silver Sulfadiazine Treatment**	0/10	0/10	0/10	0/10	0/10	0/10	0/10†	0/10

*No further deaths for 21 days postburn.
**Treatment begun at 4 hours postburn.
†$P < 0.01$ (Fisher's Exact Test).

TABLE 4

MORTALITY (NUMBER OF DEAD VERSUS NUMBER IN GROUP) IN BURNED, *Pseudomonas aeruginosa*-INFECTED (STRAIN 59-1244) MICE

Challenge Dose	DAY POSTBURN								Cumulative Mortality
	1	2	3	4	5	6	7*	8	
Burn Control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Untreated	0/10	7/10	9/10	9/10	9/10	9/10	9/10	10/10	10/10
Sulfamylon Treatment**	0/10	4/10	8/10	8/10	8/10	8/10	8/10	8/10†	8/10
Silver Sulfadiazine Treatment**	0/10	1/10	3/10	4/10	4/10	4/10	4/10	4/10†	4/10

*No further deaths for 21 days postburn.
**Treatment begun at 24 hours postburn.
†Not significant (Fisher's Exact Test).

TABLE 5

MORTALITY (NUMBER OF DEAD VERSUS NUMBER IN GROUP) IN BURNED, *Pseudomonas aeruginosa*-INFECTED (STRAIN VA-134) MICE

Challenge Dose	DAY POSTBURN							Cumulative Mortality
	1	2	3	4	5	6	7*	
Burn Control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Untreated	0/10	10/10	-	-	-	-	-	10/10
Sulfamylon Treatment**	0/10	10/10†	-	-	-	-	-	10/10
Silver Sulfadiazine Treatment**	0/10	5/10	8/10	8/10	8/10	8/10	8/10†	8/10

*No further deaths for 21 days postburn.
**Treatment begun at 24 hours postburn.
†Not significant (Fisher's Exact Test).

TABLE 6

MORTALITY (NUMBER OF DEAD VERSUS NUMBER IN GROUP) IN BURNED, *Proteus mirabilis*-INFECTED (STRAIN 77082234) MICE

Challenge Dose	DAY POSTBURN							Cumulative Mortality
	1	2	3	4	5	6	7*	
Burn Control	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10
Untreated	3/10	10/10	-	-	-	-	-	10/10
Sulfamylon Treatment**	3/10	10/10†	-	-	-	-	-	10/10
Silver Sulfadiazine Treatment**	3/10	8/10	9/10	10/10†	-	-	-	10/10

*No further deaths for 21 days postburn.
**Treatment begun at 24 hours postburn.
†Not significant (Fisher's Exact Test).

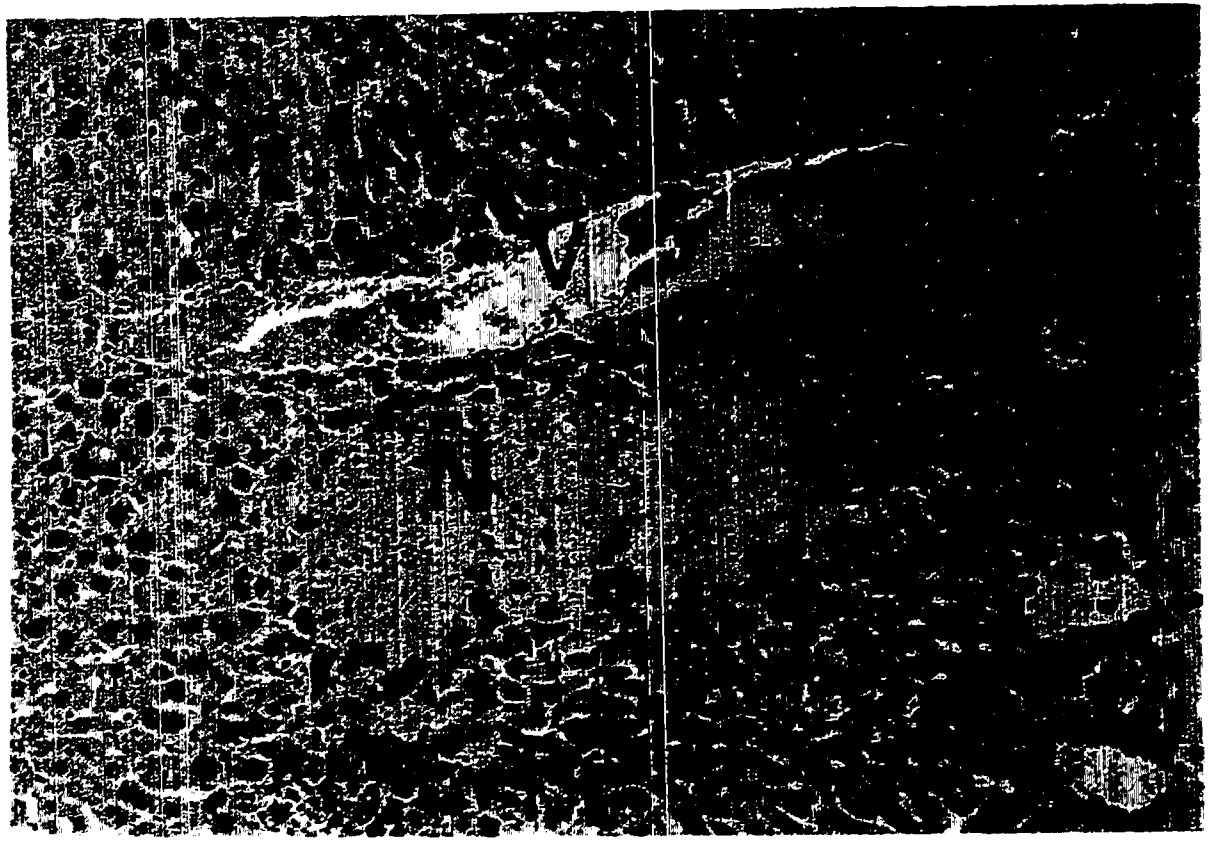


FIGURE 1. Hepatic necrosis (N) with minimal inflammatory cell infiltration in mouse treated with mafenide acetate (treatment begun four hours postburn). Note the close proximity of the necrosis to the portal veins (V) indicating hematogenous spread of Proteus mirabilis. Approximately 28 hours postburn. (H&E Stain)

silver sulfadiazine showed no histologic evidence of systemic infection.

DISCUSSION

Topical inoculation of Pseudomonas aeruginosa or Proteus mirabilis onto dorsal scalds of mice has been previously reported to cause a progressive invasive infection (11-12). Mafenide acetate and silver sulfadiazine have been shown to be effective in reducing mortality in Pseudomonas aeruginosa (Strain 59-1244) infected rats (9). These two agents are also effective against Pseudomonas aeruginosa, Strains 59-1244 and VA-134 in the mouse model. Data from the two models are qualitatively the same except that treatment must be started sooner in the mouse. In mice, treatment must be begun within four hours postburn to show efficacy. Silver sulfadiazine was effective in treating Proteus mirabilis burn wound infection in mice, but mafenide acetate was ineffective as is also true in the rat. Again, time of initiation of therapy is important. The present study has shown that the scalded mouse model may be used as an alternative to the scalded rat model in testing topical agents.

RESEARCH COMPLETION REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: A Multicenter Open Study of the Efficacy, Safety, and Tolerance of PRIMAXIN® (Previously Referred to as MK0787/MK0791 and Thienamycin Formamidine/Potentiator) in the Parenteral Therapy of Infection Caused by Pathogenic Bacteria in Hospitalized Patients

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 12 January 1986

INVESTIGATORS

Gary R. Culbertson, MD, Captain, MC
Albert T. McManus, PhD
Patrick A. Conarro, MD, Captain, MC
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: A Multicenter Open Study of the Efficacy, Safety, and Tolerance of PRIMAXIN® (Previously Referred to as MK0787/MK0791 and Thienamycin Formamidine/Potentiator) in the Parenteral Therapy of Infection Caused by Pathogenic Bacteria in Hospitalized Patients

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 12 Jan 85

INVESTIGATORS: Gary R. Culbertson, MD, Captain, MC
Albert T. McManus, PhD
Patrick A. Conarro, MD, Captain, MC
William F. McManus, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

PRIMAXIN® (imipenem-cilastatin) was examined for safety and efficacy in a population of 20 seriously burned patients with acute bacterial infections. The cohort was made up of 18 males and two females with an average age of 38 years and average burn size of 52 percent of the total body surface area. Inhalation injury was present in 14 patients. The infections treated included 16 pulmonary infections, two urinary tract infections, one wound infection, and one bacteremia. Treatment was clinically successful in 13 patients; five patients showed no improvement and the clinical response could not be determined in two patients in whom multisystem organ failure preceded the treated infection. All clinical failures were in the pulmonary infection group. No serious toxicity or side effects were noted. No patient expired while receiving the drug or as a consequence of known failure of the drug. Microbiologic success was noted in 12 patients. Resistant organisms developed in eight patients, of whom five were in the clinical failure group. Pseudomonas aeruginosa resistant to PRIMAXIN® was isolated from seven patients and occurred at an average of 3.6 days after starting treatment.

A MULTICENTER OPEN STUDY OF THE EFFICACY, SAFETY, AND TOLERANCE OF PRIMAXIN® (PREVIOUSLY REFERRED TO AS MK0787/MK0791 AND THIENAMYCIN FORMAMIDINE/POTENTIATOR) IN THE PARENTERAL THERAPY OF INFECTION CAUSED BY PATHOGENIC BACTERIA IN HOSPITALIZED PATIENTS

INTRODUCTION

Infection remains the most common cause of morbidity and mortality in severely burned patients (1). Such infection is a manifestation of injury-related immunosuppression and failures of treatment often resulting from development of microbial resistance.

Opportunistic organisms causing infections in burned patients are frequently hospital acquired and may be resistant to multiple antibiotics. The combination of host susceptibility and the possible presence of resistant organisms make the infected burn patient a chemotherapeutic challenge (2-3). The continuing accumulation of antibiotic resistant organisms in the clinical environment mandates evaluation of the effectiveness and safety of newly developed antibiotics.

We have examined the effectiveness of PRIMAXIN®, a novel thienamycin antibiotic, in a group of burn patients with serious infections. PRIMAXIN® is a combination of imipenem, the N-formimidoyl monohydrate derivative of thienamycin, and cilastatin, the sodium salt of a derivatized heptenoic acid which inhibits renal dihydropeptidase (4-5). Cilastatin increases the renal clearance of imipenem above the glomerular filtration rate, acting in a competitive and freely reversible manner to decrease renal beta-lactam hydrolysis, reduce nephrotoxicity, and increase urinary tract bioavailablity (4,6). A review of patients treated worldwide with PRIMAXIN® for a variety of bacterial infections indicates that its frequency of adverse reactions and tolerance by patients

---

[1]Pruitt BA Jr: The diagnosis and treatment of infection in the burn patient. Burns 11:79-91, 1984.

[2]Pruitt BA Jr: The burn patient. I. Initial care. Curr Prob Surg 16:1-60, 1979.

[3]Pruitt BA Jr: The burn patient. II. Later care and complications of thermal injury. Curr Prob Surg 16:1-95. 1979.

[4]Birnbaum J, Kahan FM, Kropp H, et al: Carbapenems, a new class of beta-lactam antibiotics: discovery and development of imipenem/cilastatin. Am J Med 78(Suppl 6A):3-21, 1985.

[5]Neu HC: Clinical perspectives on imipenem. J Antimicrob Chemother 12(Suppl D):149-153, 1983.

[6]Norrby SR: Imipenem/cilastatin: rationale for a fixed combination. Rev Infect Dis 7(Suppl 3):S447-S451, 1985.

parallel those of currently utilized beta-lactam antibiotics (7). This antibiotic, the first representative of a new class of beta-lactam antibiotics, the carbapenems, has a wide range of activity against most clinically important Gram-positive and Gram-negative human pathogens (8-9). Tests against more than 3,000 isolates from burn patients treated at this Institute prior to this clinical trial showed PRIMAXIN® sensitivity in more than 99 percent. A previous in vitro survey at another burn center showed sensitivity in 97 percent of tested burn isolates (10).

METHODS AND MATERIALS

Twenty patients were entered into the study. At entry, each had an acute bacterial infection with organisms susceptible to PRIMAXIN® in vitro. Infections were diagnosed using this Institute's previously described criteria (11). Inhalation injury was diagnosed by broncoscopy and/or ^{133}Xenon scan of the lungs (12-13). Microbiological effects of treatment were evaluated with daily qualitative or quantitative cultures of the infected sites and antibiotic sensitivity testing (14).

The antibiotic was administered as monotherapy at a dosage ranging from one to four grams per day, the highest doses being administered to those with the most severe infections. The clinical and bacteriologic course of each patient was followed

---

[7]Calandra GB, Brown KR, Grad LC, et al: Review of adverse experiences and tolerability in the first 2,516 patients treated with imipenem/cilastatin. Am J Med 78(Suppl 6A):73-78, 1985.

[8]Jones RN: Review of the in vitro spectrum of activiity of imipenem. Am J Med 78(Suppl 6a):22-32, 1985.

[9]Kropp H, Gerckens L, Sundelof LG, et al: Antibacterial activity of imipenem: the first thienamycin antibiotic. Rev Infect Dis 7(Suppl 3):S389-S410, 1985.

[10]Hansbrough JF, Carroll WB, Zapata-Sirvent RL, et al: Identification and antibiotic susceptibility of bacterial isolates from burned Patients. Burns 11:393-403, 1985.

[11]Shirani KZ, McManus AT, Vaughan GM, et al: Effects of environment on infection in burn patients. Arch Surg 121:31-36, 1986.

[12]Herndon DN, Thompson PB, and Traber DL: Pulmonary injury in burned patients. Crit Care Clin 1:79-96, 1985.

[13]Pruitt BA Jr, Erickson DR, and Morris A: Progressive pulmonary insufficiency and other pulmonary complications of thermal injury. J Trauma 15:369-379, 1975.

[14]McManus AT, McManus WF, Mason AD Jr, et al: Microbial colonization in a new intensive care burn unit. Arch Surg 120:217-223, 1985.

and documented, with evaluations made for bacteriologic and clinical efficacy as well as for the safety and patient tolerance of the regimen. Laboratory data were obtained before, during, and after therapy to identify hematologic, renal, and/or hepatic dysfunction.

RESULTS

The characteristics of the cohort are presented in Table 1. There were 18 males and two females in the study. Their average age was 38 years and the average burn size was 52 percent of the total body surface. Inhalation injury was present in 14 patients (70 percent). Among these 20 severely burned patients, pulmonary infections occurred in 16 (80 percent). Inhalation injury was present in 12 of the 16 patients (75 percent) with pulmonary infection. Other infections included two cases of urinary tract infection, one case of burn wound infection, and one case of bacteremia. The duration of monotherapy with PRIMAXIN® ranged from three to 19 days, the average duration of therapy being nine days. Clinical improvement or cure occurred in 13 patients (65 percent). Improvement occurred in nine cases of pulmonary infection (56 percent) and in all four of the other infections (100 percent). No improvement was noted in five patients. Clinical response was considered indeterminate in two patients. In these two patients, multisystem organ failure preceding the infection treated with PRIMAXIN® complicated the clinical evaluation of response to the drug. All clinical failures occurred in patients with pulmonary infection. Toxicity and side effects were minimal, with a transient skin rash noted at the site of infusion in one patient and transient premature ventricular contractions of one day's duration in another. No patients expired while receiving the drug or as a consequence of a known failure of the drug. Ultimately, six patients died. Of these six, two were from the clinically improved group, two from the clinical failure group, and the two patients in whom the clinical response was classified as indeterminate. These deaths occurred at an average of 33 days after PRIMAXIN® therapy was begun.

Assessment of the microbiological effects of PRIMAXIN® treatment revealed that the infecting organisms were eradicated in six patients and were reduced in number or suppressed in six patients. In eight patients, the infecting organisms developed resistance or persisted. Of the eight microbiological failures, five were associated with clinical failure. Of the five clinical failures, four had infections caused by *Pseudomonas aeruginosa* and the fifth was an infection caused by *Haemophilus parainfluenzae*. Of the eight patients in whom resistance developed, seven had infections caused by *Pseudomonas aeruginosa*. In this study, the emergence of PRIMAXIN®-resistant *Pseudomonas aeruginosa* was universal and

TABLE 1. Characteristics of Patients Entered in the PRIMAXIN[R] Study.

Patient	Age	Sex	Burn Size[a]	Inhalation Injury	Type of Infection	Organism	Dose (gm/d)	Treatment Days	Clinical Effect	Microbiology Effect	Adverse Reactions
1	36	M	56.0	Positive	Bacteremia	Enterobacter cloacae	04	11	Cured	Eradicated	None
2	36	M	79.0	Positive	Bronchopneumonia, Bacteremia	Staphylococcus aureus	04	9	Improved	Reduced in Number	None
3	20	M	81.0	Positive	Bronchopneumonia, Bacteremia	Pseudomonas aeruginosa, Staphylococcus aureus	02	6	Not Improved	Developed Resistance	None
4	29	M	82.0	Positive	Bronchopneumonia	Pseudomonas aeruginosa	04	6	Indeterminate	Developed Resistance	None
5	28	M	70.5	Positive	Bronchopneumonia	Pseudomonas aeruginosa	03	5	Not Improved	Developed Resistance	None
6	24	F	29.0	Negative	Bronchopneumonia	Haemophilus parainfluenzae	02	8	Cured	Eradicated	Premature ventricular Contractions (Slight) for One Day
7	27	M	43.0	Positive	Bronchopneumonia	Proteus vulgaris, Staphylococcus aureus	03	9	Improved	Reduced in Number, Eradicated	None
8	33	M	84.0	Positive	Bronchopneumonia	Escherichia coli	02	15	None	Reduced in Number	None
9	64	M	48.3	Positive	Bronchopneumonia	Pseudomonas aeruginosa	03	6	Improved	Persisted	None
10	55	M	54.0	Positive	Bronchopneumonia	Pseudomonas aeruginosa	02	8	Not Improved	Developed Resistance	None
11	41	M	44.0	Positive	Bronchopneumonia	Staphylococcus aureus	03	8	Cured	Eradicated	None
12	57	M	25.0	Positive	Bronchopneumonia	Pseudomonas aeruginosa	04	9	Improved	Developed Resistance	None
13	61	M	41.0	Negative	Bronchopneumonia	Enterobacter cloacae	04	10	Cured	Reduced in Number	None
14	70	M	32.0	Negative	Bronchopneumonia	Escherichia coli	04	11	Indeterminate	Eradicated	None
15	31	M	45.0	Positive	Tracheobronchitis	Streptococcus viridans, Nonhemolytic Streptococcus, Not Group D	02	12	Improved	Reduced in Number	Slight Rash at Infusion Site
16	13	M	60.0	Negative	Tracheobronchitis	Haemophilus parainfluenzae	02	3	Not Improved	Developed Resistance	None
17	52	M	56.0	Positive	Tracheobronchitis	Serratia marcescens	03	7	Improved	Eradicated	None
18	31	F	23.5	Negative	Urinary Tract	Pseudomonas aeruginosa, Enterobacter cloacae	02	9	Improved	Developed Resistance	None
19	27	M	22.0	Negative	Urinary Tract	Klebsiella pneumoniae	02	6	Cured	Suppressed	None
20	23	M	38.0	Positive	Wound	Enterobacter cloacae	03	19	Cured	Eradicated	None

[a]Percent of total body surface area burned.

occurred at an average of 3.6 days after starting the drug. Development of in vitro resistance, however, was not uniformly associated with clinical failure.

DISCUSSION

PRIMAXIN® appears to be an antimicrobial of low toxicity that is clinically effective in the treatment of infections in seriously burned patients. In this study, 80 percent of the patients had burns of more than 30 percent of the total body surface, 70 percent also had inhalation injury, and seven patients (35 percent) were over 40 years of age. Modern topical therapy limits the occurrence of burn wound infection, but such immunosuppressed patients remain uniquely susceptible to other infections.

In the absence of a concurrent control group, statistical inference concerning the effects of PRIMAXIN® on mortality in this study is inappropriate. The observed mortality in this group of patients was entirely consistent with that expected on the basis of recent experience in infected patients having injuries of this severity, indicating that PRIMAXIN® monotherapy was at least as effective as the frequently used multiple antibiotic therapeutic regimens (15-16).

PRIMAXIN® was clinically effective in most infections in this population; therapeutic failures occurred only in patients with pulmonary infections. Infections caused by Pseudomonas aeruginosa responded less favorably and were associated with rapid development of in vitro resistance; similar occurrences have been noted in patients with Pseudomonas infections in other patient studies (17-19). Adverse reactions to the antibiotic have been infrequently observed in previous studies,

---

[15] Shirani KZ, Pruitt BA Jr, and Mason AD Jr: The influence of inhalation injury and pneumonia on burn mortality. Ann Surg (in press).

[16] Mason AD Jr, McManus AT, and Pruitt BA Jr: Association of burn mortality and bacteremia: a twenty-five year review. Arch Surg (in press).

[17] Brooks RG, McCabe RE, Vosti KL, et al: Open trial of imipenem/cilastatin for serious bacterial infections. Rev Infect Dis 7(Suppl 3):S496-S505, 1985.

[18] Heseltine PNR, Yellin AE, Appleman MD, et al: Imipenem therapy for perforated and gangrenous appendicitis. Surg Gynecol Obstet 162:43-48, 1986.

[19] Nielsen DM, Katz JR, AhLoy RD, et al: Imipenem/cilastatin therapy for serious bacterial infections. Rev Infect Dis 7(Suppl 3):S506-S512, 1985.

and our experience reaffirms the safety of this antibiotic (20-23).

SUMMARY

This study indicates that monotherapy with PRIMAXIN[R] is effective and safe when used for the treatment of a wide variety of infections in patients with severe burn injury. The incidence (100 percent of all patients with Pseudomonas aeruginosa infections in this study) and rapidity (average 3.6 days after initiation of therapy) of the development of in vitro resistance to PRIMAXIN[R], however, indicates that infections in burned patients caused by Pseudomonas aeruginosa should not be treated with PRIMAXIN[R] as a single agent.

PRESENTATIONS/PUBLICATIONS

None.

---

[20]Trumbore D, Pontzer R, Levison ME, et al: Multicenter study of the clinical efficacy of imipenem/cilastatin for treatment of serious infections. Rev Infec Dis 7(Suppl 3):S476-481, 1985.

[21]Acar JF: Therapy for lower respiratory tract infections with imipenem/cilastatin: a review of worldwide experience. Rev Infec Dis 7(Suppl 3):S513-S517, 1985.

[22]Chiodini PL, Geddes AM, Smith EG, et al: Imipenem/cilastatin in the treatment of serious bacterial infections. Rev Infec Dis 7(Suppl 3):S490-S495, 1985.

[23]Kager L and Nord CE: Imipenem/cilastatin in the treatment of Intraabdominal infections: a review of worldwide experience. Rev Infect Dis 7(Suppl 3):S518-S521, 1985.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: Pharmokinetic Study of Amikacin in Burned Patients

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

James C. McKay, MD, Major, MC
Albert T. McManus, PhD
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: Pharmokinetic Study of Amikacin in Burned Patients

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: James C. McKay, MD, Major, MC
Albert T. McManus, PhD
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

A major complication of thermal injury is infection, frequently with Gram-negative organisms. Amikacin is often the drug of choice. Amikacin is excreted by the kidneys and is nephrotoxic. This study was designed to determine the proper dosage of amikacin in thermally-injured patients. We have performed the kinetic modeling phase of the experiment (drawing five blood samples after infusion of the first dose of amikacin) in six patients.

HUMAN
VOLUNTEER
DRUG
AMIKACIN
GLOMERULAR FILTRATION RATE
PHARMOKINETICS
BURNS
THERMAL INJURY

PHARMOKINETIC STUDY OF AMIKACIN IN BURNED PATIENTS

INTRODUCTION

Amikacin is frequently used in the treatment of burned patients, either to treat infections or perioperatively. During fiscal year 1982, final disposition was made on 231 patients. Of these, 131 received at least one dose of amikacin.

Several studies, including one of 10 burned patients treated with amikacin, have demonstrated that the recommended dosages of aminoglycosides frequently yield serum concentrations which are lower than the therapeutic range (1-5). However, the studies do not define the mechanism resonsible for the low serum levels. Therefore, the following study was proposed.

MATERIALS AND METHODS

A maximum of 25 patients will be studied. All patients age 16 or older are eligible for entry into the study. When the ward physician determines that a patient age 16 or older needs to be started on amikacin, one of the investigators explains this protocol to the patient or appropriate substitute for the purpose of obtaining informed consent prior to the institution of drug treatment.

After obtaining informed consent, all patients have the pharmokinetics of amikacin determined by a standard technique (1). Briefly, amikacin (3-5 mg/kg) is infused intravenously for 60 minutes. Serum samples are obtained 30 minutes and four to after infusion. Amikacin serum concentrations are then measured using an Abbott TDx drug analyzer. After the kinetic

---

[1]Zaske DE, Irvine P, Strand LM, Strate RG, Cipolle RJ, and Rotschafter J: Wide interpatient variations in gentamicin dose requirements for geriatric patients. JAMA 248:3122-3126, 1982.

[2]Zaske DE, Sawchuk RJ, Gerding DN, and Strate RG: Increased dosage requirements of gentamicin in burn patients. J Trauma 16:824-828, 1976.

[3]Zaske DE, Cipolle RJ, and Strate RG: Gentamicin dosage requirements: wide interpatient variations in 242 surgery patients with normal renal function. Surgery 87:164-169, 1980.

[4]Zaske DE, Sawchuk RJ, and Strate RG: The necessity of increased doses of amikacin in burn patients. Surgery 84:603-608, 1978.

[5]Zaske DE, Bootman JL, Solem LB, and Strate RGL: Increased burn patient survival with individualized dosages of gentamicin. Surgery 91:142-149, 1982.

infusion, amikacin is continued at a dosage determined by the patient's ward physician.

Subsequently, peak (30 minutes after an intravenous dose) and trough (immediately prior to a dose) levels of amikacin are determined once daily while the patient is receiving the drug. In addition, a 24-hour urine is collected daily for determination of drug and creatinine clearance. If toxic levels of the drug are found, the ward physician is immediately notified so that appropriate adjustments of the dosage can be made.

This protocol places no restrictions on any other therapy the patient may receive. Any decision to discontinue amikacin is made by the patient's ward physician.

The only special invasive technique (requiring only a venous blood sample) is the determination of peak and trough serum levels of the aminoglycoside since the standard procedure at this Institute is to obtain serum electrolytes and craetinine daily.

RESULTS

An Abbott TDx drug analyzer was acquired by this Institute. Amikacin levels are now determined in less than five minutes. Computer programs to calculate the pharmokinetics have been written. We have performed the kinetic modeling phase of the experiment (drawing five blood samples after infusion of the first dose of amikacin) in six patients and used the computer programs to calculate the pharmokinetics. The maximum number of 25 patients will be entered into this study within the next fiscal year.

DISCUSSION

The relationship of the pharmokinetics and renal clearance of amikacin to patient age, sex, renal function, percent initial burn, percent total body surface area not covered at the time of drug administration, weight, and percent change from preburn weight will be determined by multiple correlation and regression techniques.

PRESENTATIONS/PUBLICATIONS

None.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: Therapeutic and Prophylactic Effects of Silver-Nylon Dressings with Weak Direct Current on Experimental *Proteus mirabilis* Burn Wound Sepsis

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Chi-Sing Chu, MD
Albert T. McManus, PhD
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: Therapeutic and Prophylactic Effects of Silver-Nylon Dressings with Weak Direct Current on Experimental *Proteus mirabilis* Burn Wound Sepsis

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: Chi-Sing Chu, MD
Albert T. McManus, PhD
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

We have examined the possible therapeutic effects of silver iontophoresis from silver-coated nylon burn dressings in a rat model of fatal *Proteus mirabilis* burn wound sepsis. Therapeutic effects were tested in male Sprague-Dawley rats with 20-percent full-thickness scald wounds seeded with 10^5 colony-forming units of *Proteus mirabilis*. Constant current of 40, 60, or 90 microamperes was used. Treatment was initiated two hours after burning and seeding and was continued for five days. Survival was recorded at 21 days. The silver-nylon was therapeutic at currents between 60 to 90 microamperes ($P < 0.01$). The observed protection was also significantly better than with silver sulfadiazine.

The possible barrier effect of silver-coated nylon was also examined. Silver-coated nylon dressings were applied to burn wounds prior to the application of 10^5 colony-forming units of *Proteus mirabilis*. Inoculated animals were examined with or without applied current. Uncoated nylon was used as a control. Silver-coated nylon without current was found to be effective in preventing fatal infection because survival of greater than 90 percent was observed in all silver nylon-dressed animals. Control groups (uncoated nylon) showed greater than 70-percent mortality.

THERAPEUTIC AND PROPHYLACTIC EFFECTS OF SILVER-NYLON DRESSINGS WITH WEAK DIRECT CURRENT ON EXPERIMENTAL *Proteus mirabilis* BURN WOUND SEPSIS

INTRODUCTION

Tissue diffusibility of insoluble silver salts formed *in vivo* limits their therapeutic activity. We have examined in burned rats the possible therapeutic effects of current load-dependent silver iontophoresis from silver-coated nylon (SN) burn dressings against fatal *Proteus mirabilis* burn wound sepsis. A direct current circuit with an external SN dressing anode and a subeschar silver wire cathode was established using a constant current auto-adjusting electrical source. We have previously reported the therapeutic and prophylactic effects of this system on fatal *Pseudomonas aeruginosa* burn wound sepsis using the same rat model (1-3).

METHODS AND MATERIALS

Silver-Nylon Cloth. The cloth substrate of SN cloth is a knit nylon fabric which is available from light weaves to heavy mesh fabrics. The cloth is coated with metallic silver to achieve a very conductive yet flexible material. Based on preliminary *in vitro* experiments, Style A-2589-5, a heavy rip-stop fabric, was selected for this *in vivo* experiment (Swift Textile Metalizing Corporation, Hartford, Connecticut). The material weighs 84.8 grams per square meter. Uncoated nylon (UN) cloth of the same weave was used as a control dressing (4).

---

[1]Chu CS, McManus AT, Pruitt BA Jr, *et al*: Antibacterial effects of silver-nylon electrodes with weak direct current on *Pseudomonas aeruginosa*-infected burn wounds - a comparison with silver-sulfadiazine cream. In US Army Institute of Surgical Research *Annual Research Progress Report* for Fiscal Year 1985. San Antonio: US Government Printing Office, 1987, pp 204-214.

[2]Chu ZS, McManus AT, and Mason AD Jr: Healing effects of silver-nylon anodal dressings and weak direct current on scald injuries. *Proceedings of the First Sino-American Conference on Burn Injuries* (Abstract 64):9, 1985.

[3]Chu ZS, McManus AT, Mason AD Jr, *et al*: Histological examination of scald wound healing following treatment with silver-nylon anodal dressings and weak direct current. *Proceedings of the First Sino-American Conference on Burn Injuries* (Abstract 65):9, 1985.

[4]Roberge JK: Operational Amplifiers. Wiley and Sons, 1975, pp 452-455.

Experimental Animals. Two hundred and twenty white male Sprague-Dawley rats weighing 325 ±25 grams with 20-percent full-thickness dorsal scald burns prepared by the Walker-Mason scald technique (5) were used in this study. For infection, burn wounds were topically inoculated with Proteus mirabilis (Strain 7708234) at 10^5 organisms per rat ($\approx 1.5 \times 10^3/cm^2$). All animals were individually caged in plastic cages. Cages were insulated from contact with metal cage stands.

Direct Current Supply. A multi-channel, constant direct current generator was used to provide constant current in the range of four to 100 microamperes (μA). The simple operational amplifier circuit shown in Figure 1 converts the voltage to a current as determined by the value of R in I = V REF/R. There is a tradeoff between achieving maximum compliance and reasonable values of V REF. With an R of 10 κ ohm, the V REF of 1.0 volts. Circuit operation is maintained for load up to 125 κ ohm using ≠15-volt power supplies for typical operation amps (RC4558CN). Figure 1 also shows a microammetor in series with the load for monitoring purposes. Since the load is floating, isolation is necessary in multi-channel units. Ground-referenced loads could be driven using a Howland™ constant current source.

We modified the circuit of Figure 1 so that it provided eight channels. Isolation for metal cages was achieved using plastic cage tracks. Maximum voltage observed in this experiment was not higher than 15 volts at any amperage.

Dressing Application. At four hours or 24 hours postburn, SN or UN dressings were sutured over the infected burn wound. An electrical circuit was established by placing a 2.5 millimeter by 10 centimeter silver needle under the burn wound at the subpannicular carnosus space. In this configuration, polarity of the dressing or the needle could be established by switching electrode connections. Following suturing of the dressing, three layers of gauze and a layer of sponge with a small polyethylene irrigation tube attached were placed under the dressing. The gauze was then fixed with a flexible tubular bandage. To prevent the rat from gnawing the electrode wires and irrigation tube, the wires and tube were passed through a hole cut in a wooden tongue blade and a four-inch length, meshed wire insulator. The blade was then sutured to the back over the dressings. Animals were thus free to move in their cages but were denied access to the wires and tubes. The gauze was then moistened with two to three milliliters of saline through the irrigation tube, which was repeated two or three times per day when current was being applied (1).

---

[5]Walker HC and Mason AD Jr: A standard animal burn. J Trauma 8:1049-1051, 1968.

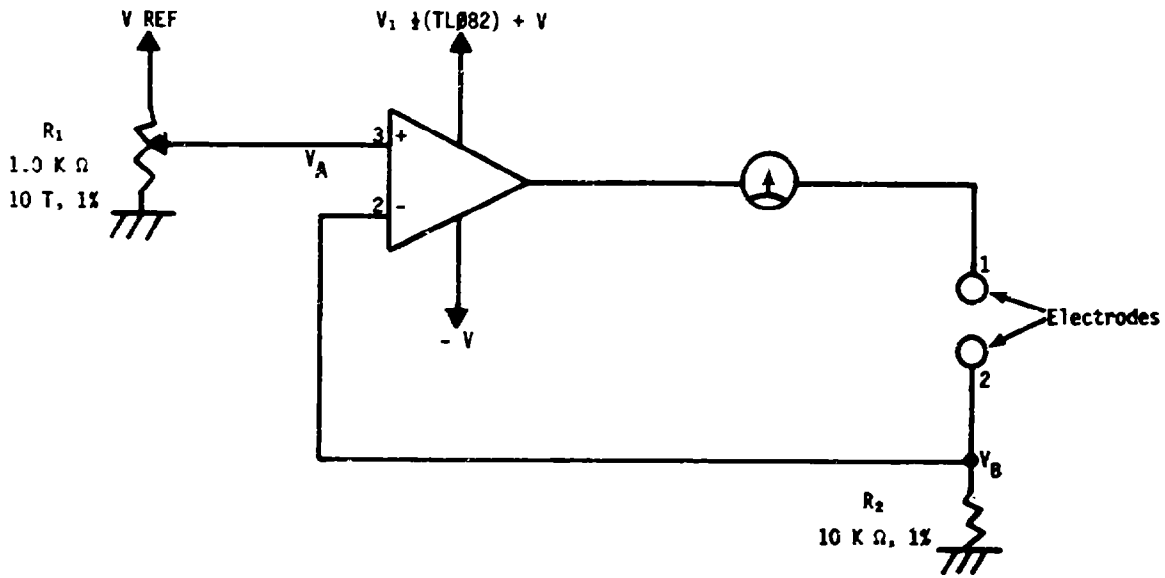


FIGURE 1. Voltage-controlled current source diagram.

Experimental Therapy. The possible therapeutic effect of anodal silver dressings was examined in 140 animals. These animals were divided into seven control and six treatment groups. The description of the control and treatment groups is given in Table 1. Silver sulfadiazine cream, when applied, was one treatment per day for five days. Mortality was recorded at 21 days.

Experimental Prophylaxis. The possibility that SN would act as a barrier to surface contamination was examined in 80 animals. These control and treatment groups are described in Table 2. After suturing the SN or UN to the burn wound, a gauze sponge containing 10^5 organisms in saline was placed on the dressing. These dressings were then covered with gauze. An irrigation tube was placed and covered with a flexible tubular bandage. Current, when applied, was for five days. Mortality was recorded at 21 days.

Statistical Analysis. Data were analyzed as multiway frequency tables with Chi-square, Yates-corrected Chi-square, or Fisher's exact test comparisons.

RESULTS

SN was found to be an effective therapeutic antimicrobial agent for Proteus mirabilis burn wound infection in proportion to the intensity of the applied amperage. Data are presented in Table 3. SN without applied current (Group 3) shows no improved survival even when compared to the untreated group (Group 2). Animals treated with low amperage (Group 5, 40 μA ($\cong$ 0.6 μA/cm^2)) showed no significant improvement. Intensities of 60 μA ($\cong$ 1.0 μA/cm^2) and 90 μA ($\cong$ 1.5 μA/cm^2) in Groups 6 and 7 showed significant therapeutic activity when compared to the untreated group ($P < 0.01$). Silver sulfadiazine showed therapeutic advantage over the 60 and 90 μA groups.

SN was also found to be an antimicrobial barrier. Group identifications are the same as listed in Table 2. Mortality results are presented in Table 4. SN was an effective antimicrobial barrier with and without applied current. However, the 4 and 40 μA amperage groups showed even higher survival rates.

DISCUSSION

The loss of the physical and immunological barriers of the skin following thermal injury is an obvious example of trauma-related immunosuppression. The susceptibility of burn wounds to infection is a major liability and topical application of antimicrobial agents has become common. Silver in the form of silver sulfadiazine or nitrate salt is the most

TABLE 1

EXPERIMENTAL THERAPY CONTROL AND TREATMENT GROUPS

Group Number	Description	Number of Rats
CONTROL GROUPS		
1	Burned Only	20
2	Burned + Infected	20
3	Burned + Infected + SN	20
4	Burned + Infected + Silvadene^{R} (2 Hours)	20
TREATMENT GROUPS		
5	Burned + Infected + SN Anode (40 μA/2 Hours)	20
6	Burned + Infected + SN Anode (60 μA/2 Hours)	20
7	Burned + Infected + SN Anode (60 μA/2 Hours)	20

TABLE 2

EXPERIMENTAL PROPHYLAXIS CONTROL AND TREATMENT GROUPS

Group Number	Description	Number of Rats
CONTROL GROUP		
8	Burned Only + UC + Infected	20
TREATMENT GROUPS		
9	Burned + SN + Infected + 4 μA (2 Hours)	20
10	Burned + SN + Infected + 40 μA (2 Hours)	20
11	Burned + SN + Infected	20

( ) indicates time postinfection that treatment was begun.
UC = Uncoated Nylon
SN = Silver-Nylon

TABLE 3

EXPERIMENTAL THERAPY MORTALITY DATA

Group Number	POSTBURN DAY																					Cumulative Mortality	Percent Mortality
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/20	0
2	-	-	11	2	3	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	17/20	85
3	-	3	7	5	1	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-	-	18/20	90
4	-	1	3	5	-	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14/20	70
5	-	-	1	7	-	9	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18/20	90
6	-	1	-	3	4	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	10/20	50
7	-	3	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7/20	35

TABLE 4

EXPERIMENTAL PROPHYLAXIS MORTALITY DATA

Group Number	POSTBURN DAY																					Cumulative Mortality	Percent Mortality
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
8	-	-	5	9	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15/20	75
9	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/20	10
10	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2/20	10
11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0/20	0

common topical agent used in the treatment of burn wounds. The antimicrobial effect of silver nitrate as a 0.5-percent solution is limited to wounds with low levels of bacterial contamination and has little value in established burn wound infection. This surface effect is probably due to the limited tissue penetration of the silver ions. The combination of the two antimicrobial agents, silver and sulfadiazine is the more commonly used form of silver. However, sulfonamide-resistant organisms show less sensitivity to silver sulfadiazine despite *in vitro* sensitivity. The mechanism of clinical resistance has not been established, but it seems likely that the resistant strains are sulfonamide-resistant through they reflect an *in vitro* reaction to the silver component. In the presence of sulfonamide resistance, the limited penetration of silver may be the clinically limiting factor.

These results indicate that SN may be useful both in preventing and, when used in conjunction with low amperage direct current, in treating burn wound infection.

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DAOG6969	2. DATE OF SUMMARY: 86 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 85 10 01	4. KIND OF SUMMARY: D	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DISP'N INSTR'N: CX	9. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BD	302
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY87-01		

11. TITLE *(Precede with Security Classification Code)* (U) The Study of Metabolism and Nutritional Effects of Burn Injury in Soldiers

12. SUBJECT AREAS
06 05 Clinical Medicine 06 13 Microbiology 06 15 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
76 10	CONT	DA	C

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED APPROVED BY

a. DATE EFFECTIVE	b. CONTRACT/GRANT NUMBER	c. TYPE	d. AMOUNT	e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
86	1.0	150
87	3.5	245

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: MASON, A D Jr
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-7832
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR *(If available)* g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Nitrogen Balance; (U) Burn Injury; (U) Computer Surveillance; (U) Mitochondria; (U) Volunteers; (U) Lab

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

22. (Continued) (U) Animals: (U) Rats; (U) Beta Oxidation; (U) RAII

23. (U) To identify the cellular mechanisms of postburn hypermetabolism and altered thermoregulation in burned soldiers and establish nutritional requirements following thermal injury.

24. (U) Studies of postburn metabolism and its mechanisms have been executed in several experimental animals. Of these, the most satisfactory is the rat, in which it is possible to study some of the possible afferent mechanisms of postburn hypermetabolism discretely. On the basis of findings in this model, we will explore the contribution of muscle activity to hypermetabolism in patients.

25. (U) 8510 - 8609. To facilitate further human studies exploring the role of muscle activity in postburn hypermetabolism, a contract has been initiated for the renovation and modernization of the controlled environmental metabolic room on Ward 14A. This work will be initiated upon completion of the room.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DAOG6968	2. DATE OF SUMMARY: 86 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 85 10 01	4. KIND OF SUMMARY: D	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING	8. DISB'N INSTR'N: CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BD	303
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP	FY87-01		

11. TITLE *(Precede with Security Classification Code)*
(U) Alteration of Host Resistance in Burned Soldiers

12. SUBJECT AREAS
06 05 Clinical Medicine 06 13 Microbiology 06 15 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
76 10	CONT	DA	C

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED APPROVED BY: Basil A. Pruitt
a. DATE EFFECTIVE
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
86	5.0	278
87	5.0	165

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: MC MANUS, A T
d. TELEPHONE NUMBER *(include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(include area code)*: 512-221-3411
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR *(if available)* g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Tissue Spreading Factors; (U) Infection; (U) Immunostimulants; (U) Virulence Factors; (U) Plasmids; (U) Antibiotic

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

22. (Continued) Effects; (U) Volunteers; (U) RAII

23. (U) To define the microbial basis of opportunistic infection in susceptible burned soldiers, identify specific mechanisms of decreased host resistance that are targeted by opportunistic pathogens, and develop and evaluate countermeasures.

24. (U) The high susceptibility of burned rats to experimental infection with Pseudomonas aeruginosa and Proteus mirabilis will be investigated. The effect of in vitro alterations of specific microbial characteristics on infection will be investigated. Specific antimicrobial and immunostimulator therapies will be examined.

25. (U) 8510 - 8609. The clinical trial of the parenteral antibiotic imipenem-cilastatin sodium (PRIMAXIM®) was completed during this reporting period. No serious adverse reactions were observed in the 20 patients studied. The antibiotic was clinically effective for most infections treated; in vitro resistance, however, developed in seven Pseudomonas aeruginosa infections. Further examination of mechanisms of antimicrobial activity of silver sulfadiazine have shown that commonly reported agar diffusion tests for demonstation of sensitivity to the compound are principally measurements of the activity of the sulfadiazine component. Agar well diffusion zones can be predicted with cheaper and standardized tests for sulfonamide sensitivity.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE. U.S. G.P.O. 1984-421-646/17001

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Albert T. McManus, PhD
Virginia C. English, MS
Camille L. Denton, MA
George T. Daye, Jr., MS
Charles H. Guymon, MS, SGT
Aldo H. Reyes, SSG
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: Albert T. McManus, PhD
Camille L. Denton, MA
Virginia C. English, MS
George T. Daye, Jr., MS
Charles H. Guymon, MS, SGT
Aldo H. Reyes, SSG
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

A clinical trial of the parenteral antibiotic ceftazidime (FORTAZ^{R}) as monotherapy in infected burn patients is in progress. Isoelectric focusing techniques to identify specific and chromosomal beta-lactamase activities of multiply resistant Gram-negative burn patient isolates have been developed. A standard set of known plasmid-mediated beta-lactamase genes has been established. A probe to detect one of the two common plasmid-mediated sulfonamide resistance genes has been developed. The second probe is expected to be finished in the next reporting period.

EXPERIMENTAL PARENTERAL AGENTS

Measurement of *in vitro* sensitivity to the investigational cephalosporin class antibiotic cefsulodin sodium (Abbott-46811) for the seventh reporting period. In 492 tested isolates of *Pseudomonas aeruginosa*, 48 resistant strains were found. A comparison of these results to previous findings is presented in Table 1. The licensing data for the drug has not been released.

The investigational antibiotic impenem-cilastatin sodium (PRIMAXIN®, Merck Sharp & Dohme Research Laboraties) was approved by the Food and Drug Administration during this reporting period. The clinical trial with this drug at this Institute was completed. A total of 20 patients were enrolled and the results were submitted for publication. *In vitro* testing of the drug was completed for 2,225 isolates. Resistance was found in 92 isolates (41 percent). *Staphylococcus aureus* isolates resistant to oxacillin were not tested for activity against imipenem-cilastatin sodium or other beta-lactam antibiotics due to the Food and Drug Administration requirement for reporting all oxacillin-resistant *Staphylococcus aureus* strains resistant to beta-latam antibiotics.

Three additional investigational drugs were examined. The experimental cephalosporin class antibiotics ceftazidime and ceftriaxone were explored for activity against burn patient isolates. Again oxacillin-resistant *Staphylococcus aureus* were excluded. Ceftazidime was found to be active in 93 percent (928/991) of tested strains. Ceftriaxone was active in 83 percent (776/993) of tested strains. Aztreonam, a monobactam antibiotic, was tested against 721 Gram-negative fermentative aerobic organisms. The activity of this new antibiotic was a very impressive 99.8 percent (720/721). Summary data for these experimental parenteral antibiotics are presented in Table 2.

EXPERIMENTAL TOPICAL AGENTS

Five-percent mafenide acetate was examined for *in vitro* activity against *Pseudomonas aeruginosa* burn patient isolates. Agar dilution minimal inhibitory concentration assays were used for 189 strains. Strains were selected on the basis of antibiotic sensitivity patterns. Each distinct antibiotic sensitivity pattern found on each patient was tested. The mean minimal inhibitory concentration was 0.246 grams per 100 milliliters and median minimal inhibitory concentration was 0.3125 grams per 100 milliliters. This was unchanged from the

TABLE 1

CEFSULODIN SODIUM ACTIVITY AGAINST BURN PATIENT *Pseudomonas aeruginosa*

	Fiscal Year 1981	Fiscal Year 1982	Fiscal Year 1983	Fiscal Year 1984	Fiscal Year 1985	Fiscal Year 1986
Resistant	8 (1.4)	143 (17.9)	49 (12.1)	36 (7.8)	59 (10.3)	48 (9.7)
Sensitive	556	655	355	463	571	444

( ) = Percent Resistant

TABLE 2

ACTIVITY OF EXPERIMENTAL ANTIBIOTICS FOR FISCAL YEAR 1986

	Ceftazidime[1]	Ceftriaxone[1]	Aztreonam[2]
Resistant	63 (6.4%)	157 (16.8%)	1 (0.01%)
Sensitive	928	776	721

[1]Against all flora except oxacillin-resistant Staphylococcus aureus.
[2]Against Gram-negative aerobic flora.

( ) = Percent Resistant

previous reporting period. Data comparing results for fiscal years 1985 and 1986 are shown in Table 3.

TABLE 3

MINIMAL INHIBITORY CONCENTRATION FOR
Pseudomonas aerginosa STRAINS TO MAFENIDE ACETATE

Mafenide Acetate Concentration (g/100 ml)	Number of Strains Fiscal Year 1985	Number of Strains Fiscal Year 1986
0.019	2	6
0.039	22	9
0.078	53	28
0.156	56	47
0.312	81	83
0.625	58	15
1.250	-	1
TOTAL NUMBER OF STRAINS	272	189

SEROLOGIC TYPES OF Pseudomonas aeruginosa ISOLATED FROM BURN PATIENTS

Pseudomonas aeruginosa isolates from 51 patients were serotyped using the Difco International Typing Sera™ set and autoclaved bacterial suspensions. Stains were selected on the basis of having a distinct antibiotic sensitivity pattern for each patient. A total of 189 strains were typed. Data are presented as the total number of patients with each serotype

and the total number of isolates per serotype in Figure 1. Serotype 11 was the predominant type identified.

TECHNIQUES TO IDENTIFY SPECIFIC PATHOGENIC MECHANISMS

Efforts to develop methods to identify specific burn pathogen virulence and resistance mechanisms have continued. In addition to previously described Gram-negative plasmid transfer techniques, agarose gel sizing, and endonuclease mapping of plasmids, methods for specific identification of plasmid mediated beta-lactamase gene products have been developed. Using isoelectric focusing methods, beta-lactamase enzymes can be identified by comparing isoelectric points of unknown plasmid mediated genes to known beta-lactamses. An example of a typical preparation containing focused beta-lactamase activities (developed with the chromogenic substrate nirocefin) of burn patient organism and control enzymes is show in Figure 2. This method adds an additional tool for identification of specific traits associated with burn pathogens.

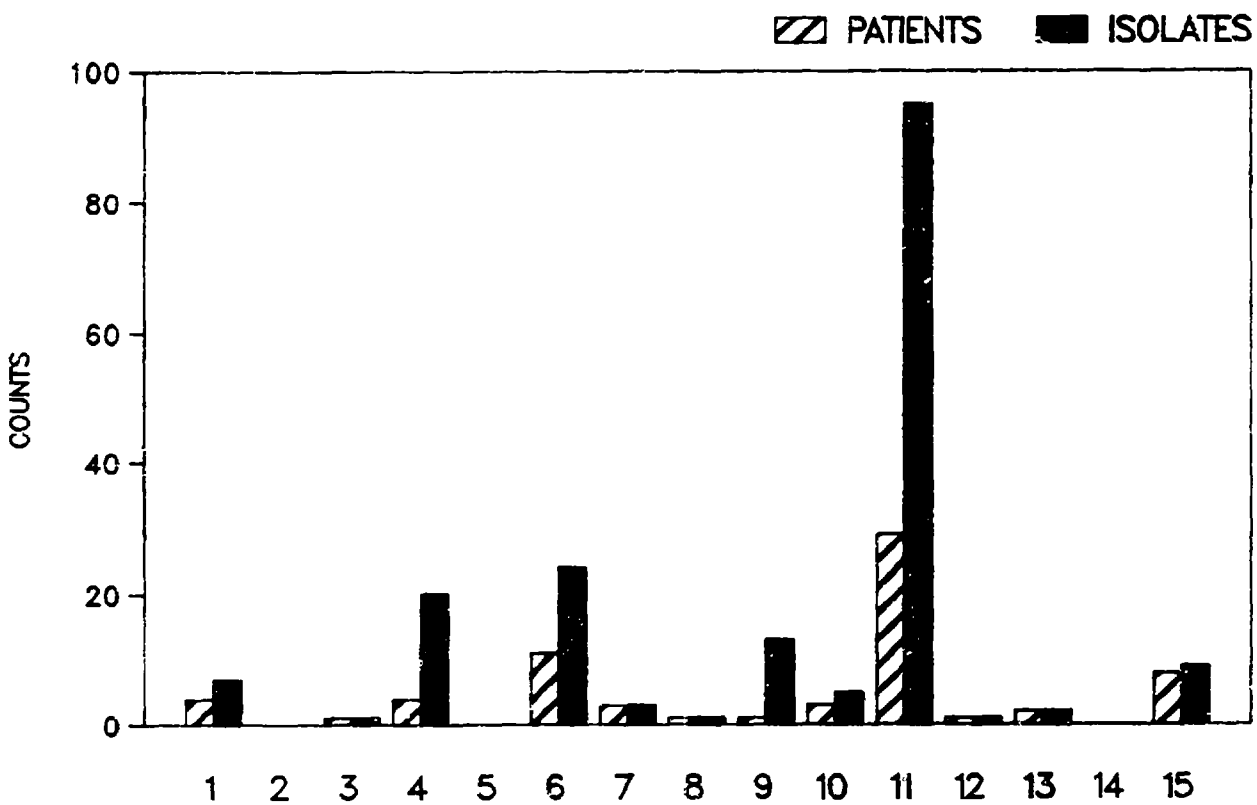


FIGURE 1. Number of patients with each serotype and isolates per serotype.

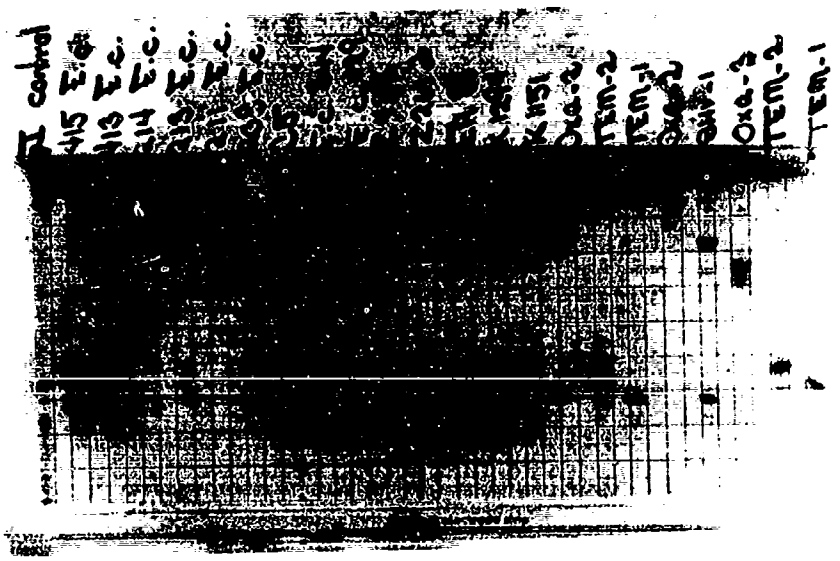


FIGURE 2. Activities of isoelectric point-focused beta-lactam enzymes.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS: Characterization of Biochemical Indicators of Infection in the Thermally Injured

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

David G. Burleson, PhD, Lieutenant Colonel, MS
Kuang-Tzu D. Lin, MD, PhD
Avery A. Johnson, BS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS: Characterization of Biochemical Indicators of Infection in the Thermally Injured

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: David G. Burleson, PhD, Lieutenant Colonel, MS
Kuang-Tzu D. Lin, MD, PhD
Avery A. Johnson, BS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

We have characterized the biochemical substances that appear in the blood of burned-infected rats and burned patients. The previously described factor with maximum 420 nanometers emission at 355 nanometers excitation was found to consist of several fluorescent substances that could be resolved by high pressure liquid chromatography on a C-18 chromatography column. Four of these fluorescent substances could be identified consistently in the sera of severely burned patients. One of the factors had a high pressure liquid chromatography retention time identical to that of neopterin and was purified seven-fold by ion exchange chromatography. Although the partially purified factor was chromatographically and spectrally similarly to neopterin, its identity could not be verified by thermospray mass spectrometry. Larger quantities of more highly purified material may be necessary to establish the identity of these fluorescent factors.

CHARACTERIZATION OF BIOCHEMICAL INDICATORS OF INFECTION IN THE THERMALLY INJURED

INTRODUCTION

Infection poses a serious threat to all severely burned patients and is a continuous obstacle to effective therapy. Timely diagnosis of sepsis can be critical to administration of therapy and patient survival. The metabolic changes induced by burn injury hamper the detection of sepsis and make objective diagnosis more difficult. Abnormal levels of hormones (1-2), acute phase proteins in serum (3-4), and fluorescent substances (5-6) in blood and plasma have been associated with the presence of inflammation and/or infection in human burn patients and animal burn models. The presence of these substances in blood and plasma probably reflect metabolic responses to stress, trauma, and infection in the host. A specific measure of invading pathogens is desirable, but the spectrum of microbes that would have to be detected at the low levels present in blood in the early phases of infection limit the general applicability of microbe-specific indicators. A biochemical measurement of specific metabolic products of the immune response against an infectious challenge would have the advantage of differentiating the metabolic response induced by trauma from the metabolic response induced by activation of the immune system. Such a test might alert the clinician to a

---

[1] Becker RA, Vaughan GM, Goodwin CW Jr, et al: Interactions of thyroid hormones and catecholamines in severely burned patients. Rev Infect Dis 5:S908-S913, 1983.

[2] Wilmore DW: Hormonal responses and their effect on metabolism. Surg Clin N Am 56:999-1018, 1976.

[3] Pepys MB and Baltz ML: Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. Adv Immunol 34:141-212, 1983.

[4] Burleson DG, Lin KD, and Powanda MC: Indicators of infection in burn patients. Proceedings of the Sixteenth Annual Meeting of the American Burn Association (Abstract 34), 10-13 April 1984.

[5] Powanda MC, Dubois J, Villarreal Y, et al: Detection of potential biochemical indicators of infection in the burned rat. J Lab Clin Med 97:672-679, 1981.

[6] Powanda MC, Dubois J, and Villarreal Y: Monitoring and modifications of the metabolic and physiologic alterations associated with thermal injury in burned soldiers. US Army Institute of Surgical Research Annual Progress Report for Fiscal Year 1981, pp 342-352, 1982.

potential problem in a more timely and objective manner than any currently available technique.

Several attempts are ongoing to find clinically useful indicators to use as adjuncts to standard microbiological methods of assessing the presence of sepsis (5-7). We have purified and attempted further characterization of the nature of the previously reported fluorescent substances found in the blood of burn victims.

MATERIALS AND METHODS

Measurement of Fluorescent Indicators. One milliliter (ml) of anti-coagulated blood was mixed with four ml cold (4° C) perchloric acid (0.8 molar (M)). After incubating for 10 minutes, the mixture was centrifuged at 4° C for 10 minutes at 3,000 g. The supernatant was recentrifuged at 20,000 g for 30 minutes. The clear supernatant was transferred to another tube and fluorescence was then measured using an Aminco-Bowman (Silver Spring, Maryland) spectrofluorometer at excitation of 355 nanometers (nm) and emission of 420 nm (355 ex/420 em). The fluorometer was standardized by using a calibration standard (fluorescence intensity block).

High Pressure Liquid Chromatography (HPLC) Determination of the 355 ex/420 em Factor in Serum. Serum (100 microliters (1)) was deproteinized by incubating at 100° C in an oil bath for 20 minutes after the addition of 200 1 of 0.2 M potassium phosphate buffer (pH = 4.5). The mixture was then centrifuged at 20,000 g for 20 minutes and the supernatant was injected directly on HPLC. HPLC was performed on a Hewlett-Packard Model 1090 liquid chromatograph with a Biophase ODS reverse phase 4.6 X 250 millimeter column (Bioanalytical Systems). The mobile phase consisted of .05 M ammonium acetate at pH 7.0. The column temperature was maintained at 45° C and the flow rate was 1.0 ml per minute. The HPLC was equipped with a Kratos fluorescence detector (Model 980) with a 25 1 flow cell. The excitation monochronometer was set at 350 nm and the emission cutoff filter was at 389 nm. The retention time for standard pterins (Sigma, St. Louis, Missouri) were determined using 10 1 of a standard solution of pterins (10 nanograms per milliliter (ng/ml)). The amount of each fluorescent substance present was measured on a Hewlett-Packard Model 3392A integrator.

---

[7]Burleson DG, Lin KD, and Powanda MC: Characterization of biochemical indicators of infection in the thermally injured. US Army Institute of Surgical Research Annual Progress Report for Fiscal Year 1984, pp 200-208.

Purification of the Indicator by Ion Exchange Chromatography. A pool of human burn serum (15 ml) known to be high in fluorescence was subjected to heat denaturation and purification of the fluorescent material by ion exchange chromatography following the method of Rothler and Karobath (8). Briefly, the boiled serum supernatant was first added to a Dowex-50 WX8 column, washed with water, and eluted by 1N ammonium hydroxide. The eluent was then added to a Dowex-1 column, washed with aqueous ammonia, and eluted with formic acid. The eluate was dried under vacuum and reconstituted in the appropriate buffer for analysis.

Mass Spectral Analysis of the 355 ex/420 em Factor. Mass spectral analysis of the ion exchange purified material was performed by Christina Hsich Vestal of the Vestec Corporation. The mass spectral analysis was performed on a Hewlett-Packard Model 5970 with a thermospray LC-MS interface (Vestec Corporation). The HPLC column was a C-18 reverse phase, the flow rate was one ml per minute, and the elution buffer was 0.1 M ammonium acetate buffer.

RESULTS

Optimal chromatographic conditions described in the MATERIALS AND METHODS section were determined for chromatography of the 355 ex/420 em factor and several pterins. The chromatograph obtained for the pterins under these conditions is shown in Figure 1. Under these conditions, neopterin had a retention time of 5.4 minutes, iso-xanthropterin 8.3 minutes, and biopterin 9.4 minutes. A patient sample which registered a relatively high fluorescence at 355 ex/420 em was deproteinized and run on HPLC. The chromatogram obtained is compared with a sample extracted from a normal control in Figure 2. Three of the peaks in the patient sample had retention times similar to neopterin, iso-xanthropterin, and biopterin, respectively (Figure 1), while none of the same peaks were present in the control sample.

To further characterize the nature of the unknown material, 15 ml of human burn patient serum was pooled and the serum protein denatured by heat. The fluorescent material remaining in the supernatant after centrifugation was purified using a modified ion exchange procedure for the purification of pterins. A HPLC chromatogram of the purified serum components is compared with one of unpurified serum in Figure 3. The peak integral value for each of the peaks remaining after

---

[8]Rothler F and Karobath M: Quantitative determination of unconjugated pterins in urine by gas chromatography/mass fragmentography. Clin Chim Acta 69:457-462, 1976.

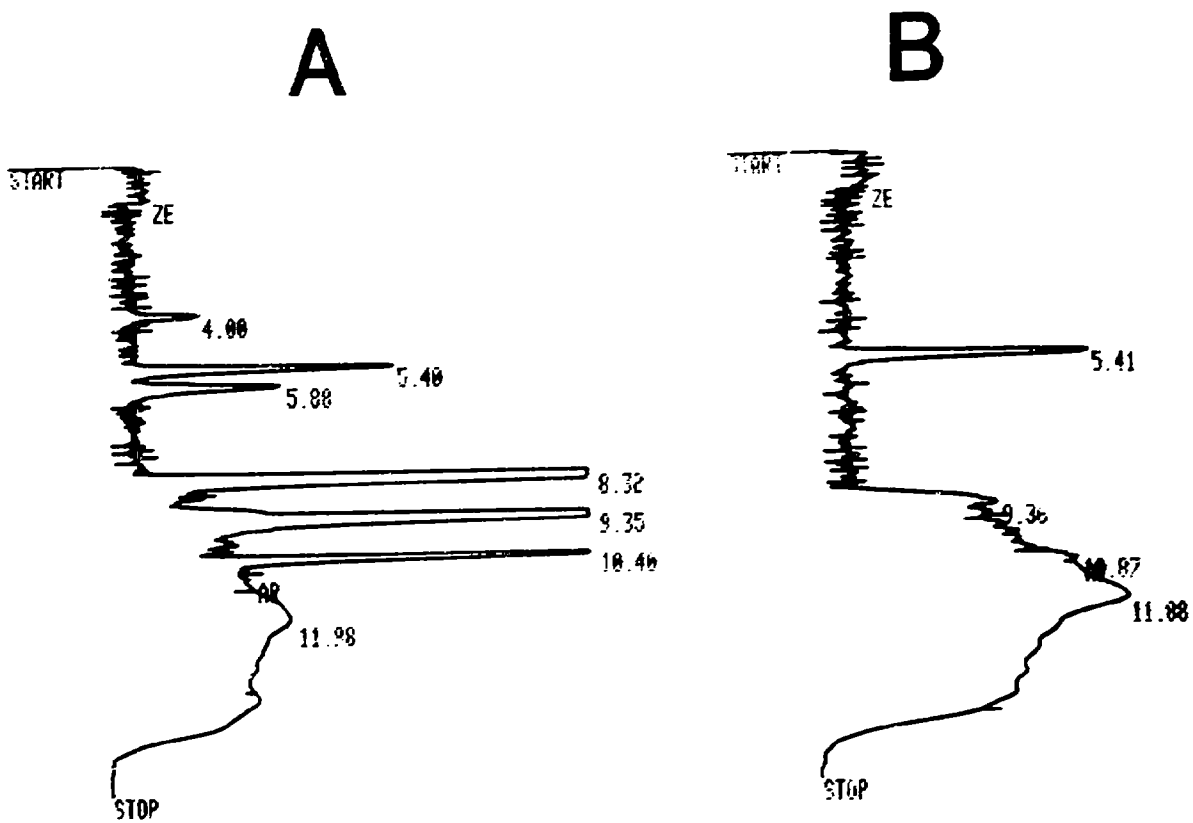


FIGURE 1. Chromatography of neopterin and five other pterins. A five-µl aliquot of a standard solution containing 10 µg/ml of pterin-6-carboxylic acid (4.0 minutes), neopterin (5.4 minutes), xanthropterin (5.88 minutes), iso-xanthopterin, biopterin (9.35 minutes), and 6-methyl pterin (10.4 minutes) was chromatographed under the conditions described in methods. A = neopterin standard alone and B = neopterin and five other pterins.

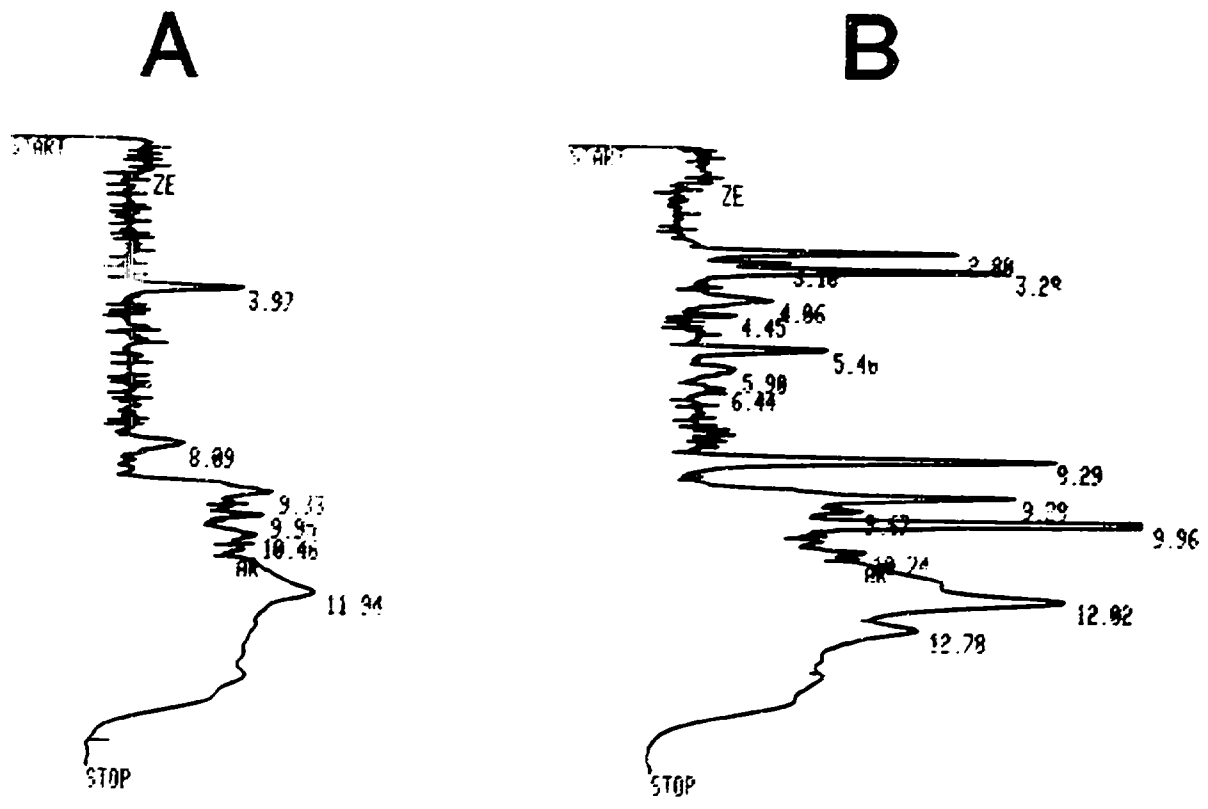


FIGURE 2. Comparison of chromatograms from a highly fluorescent patient sample and a normal control. Chromatography conditions were as described in MATERIALS AND METHODS section of the text. A = patient sample and B = control sample.

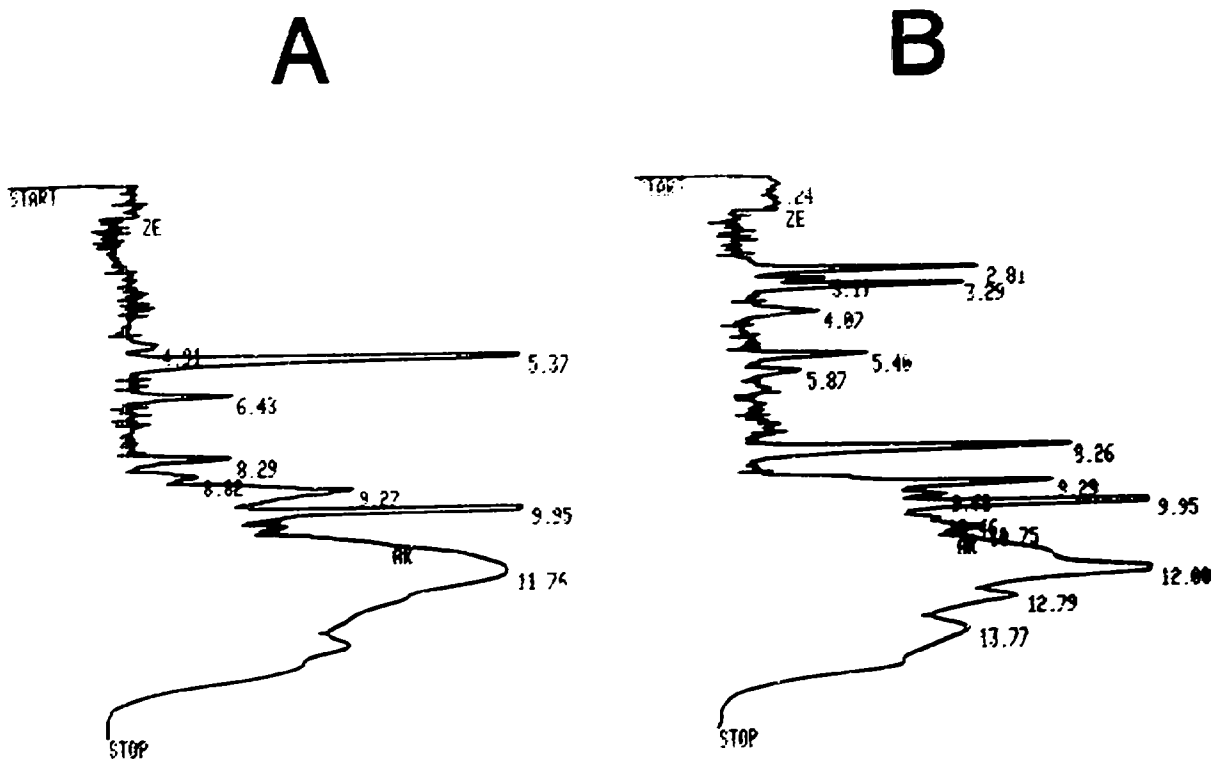


FIGURE 3. Comparison of pooled fluorescent patient sera before and after purification by ion exchange chromatography. A pool of several patient sera with a high level of 355 ex/420 em factor was purified as described in methods. A portion of the pooled sera was chromatographed before and after purification for comparison. A = pooled sera before purification and B = purified sample eluted from ion exchange column.

purification were compared to the peak integral value of fluorescent material present before purification. The peak at 5.4 minutes was selectively purified by 7.2-fold compared to the total fluorescence in the sample. The peaks at 9.3 and 9.95 minutes retained their relative concentrations after purification. The amount of the 5.4-minute material present correlated to 0.948 micrograms per ml of neopterin standard.

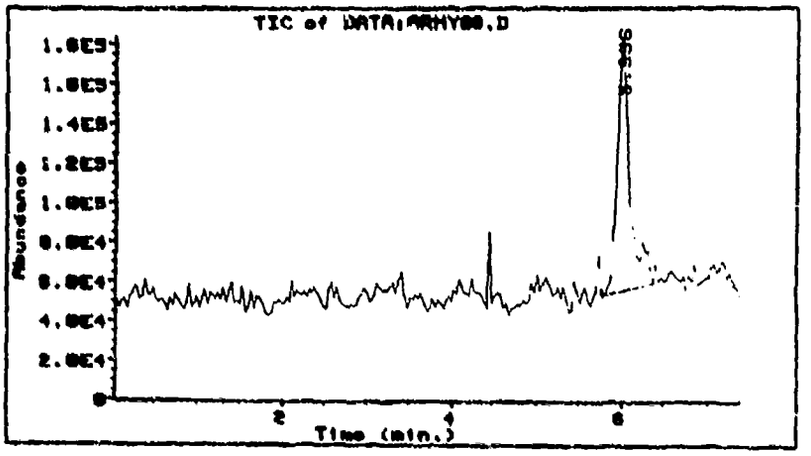
Mass spectral analysis was performed on the partially purified sample in order to try to verify the identity of the peak at 5.4 minutes. Although the optical and fluorescence spectral characteristics, chemical behavior, and HPLC chromatography of this material were similar to neopterin, confirmation of the identity as neopterin by comparison of the unknown peak with that of a known standard was unsuccessful. Since there is no fluorescence detection on the mass spectrometer, the substances separated on the HPLC column were detected by monitoring the ion fragements produced in the mass spectrometer (Figure 4).

There was relatively little material detected by total ion count monitoring at the retention time corresponding to neopterin. The amount of unknown introduced into the mass spectrometer (based on fluorescence equivalence) was equal to two ng of neopterin which is two percent of that shown in Figure 4B. This level was close to the lower detection limit for neopterin. The presence of so much nonfluorescent material may have obscured the presence of neopterin in the patient sample. Specific monitoring for the parent ion of neopterin (m+1, 254 atomic mass unit (amu)) and the principal ion fragments (192 and 218 amu) also failed to reveal the presence of neopterin (Figure 5).

Mass spectrometry analysis did reveal a substance of molecular weight of 186 amu at 0.5 minutes before the retention time of the neopterin standard. A chemical composition of the 186 amu peak could not be obtained due to the high background levels in the sample, which prevented the determination of an accurate isotopic abundance ratio for the m+2 and m+3 peak. Since HPLC colomns can vary slightly, it is not clear whether the 186 amu peak is the same as that corresponding with a neopterin retention time in HPLC with fluorescence detection. As is common with thermospray techniques, the limited fragmentation pattern revealed little of other structural clues to the structure of the 186 amu peak.

In addition to the fluorescent peak with a retention time similar to neopterin, three other fluorescent substances are present consistently when the perchloric acid supernatant is highly fluorescent at 355 ex/420 em. The chromatogram for several patients with high levels of 355 ex/420 em factor are shown in Figure 6. Neopterin has a retention time of 5.3

A



B

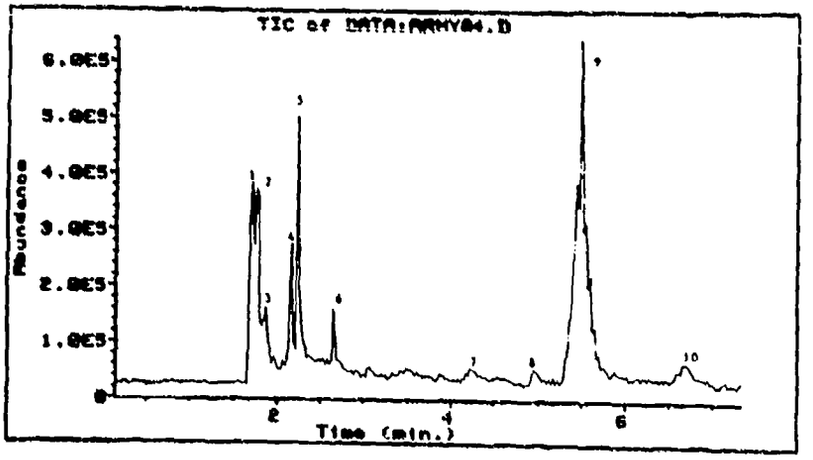


FIGURE 4. Comparison of chromatograms of purified patient sera and standard neopterin using mass spectral total ion count (TIC) for sample detection. The partially purified patient sample was chromatographed by high pressure liquid chromatography and the effluent monitored with a mass spectrometer. TIC of each substance emitted from the column is shown relative to the TIC of 100 ng standard neopterin chromatographed under the same conditions. Note the relatively small amount of material with a retention time in the range of neopterin. A = partially purified patient sample and B = neopterin standard.

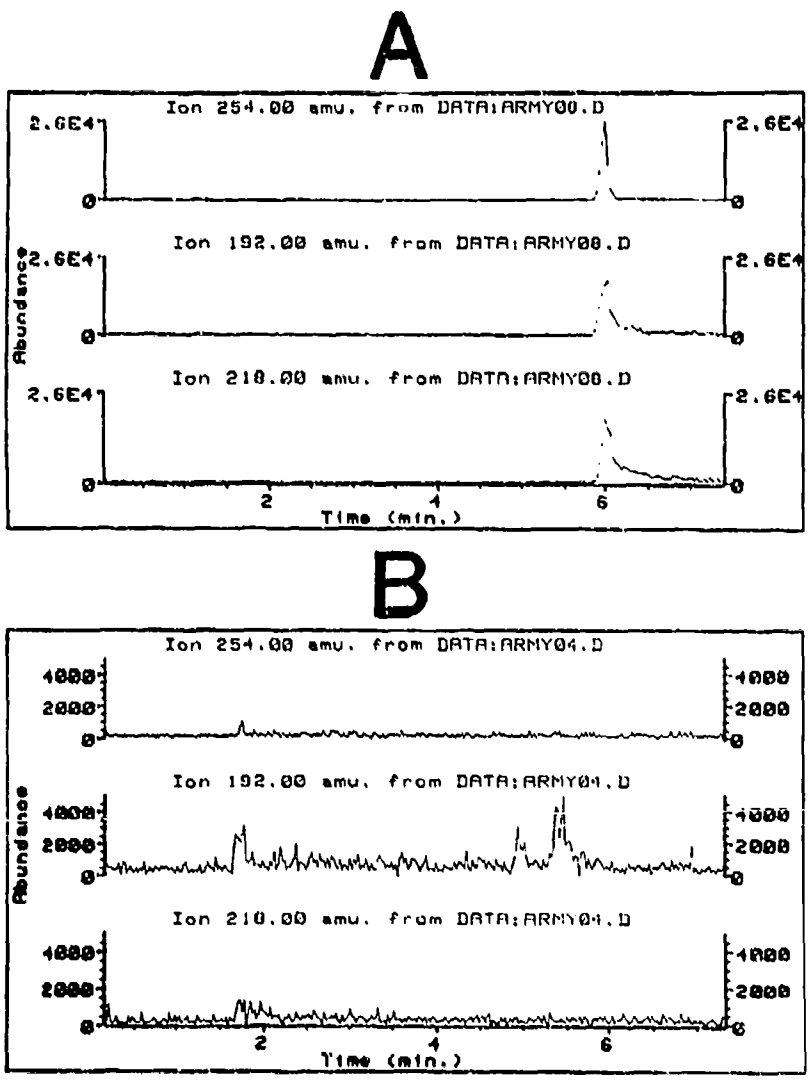


FIGURE 5. Comparison of HPLC chromatograms of partially purified patient sera and standard neopterin using mass spectral single ion monitoring for detection. Effluent from HPLC separation of the ion exchange purified patient sera was directed into the thermospray module of a mass spectrometer. The parent (M+1 = 254 amu) and major fragmentation ions (218 and 192 amu) for standard neopterin were monitored specifically to detect neopterin. The amount of fluorescent material at the neopterin retention time based on calculations of neopterin fluorescence was approximately two percent of that of the standard shown in the single ion monitor chromatogram. A = standard neopterin and B = purified pooled sera. **NOTE:** No neopterin specific peaks are visible above background in the purified sera sample.

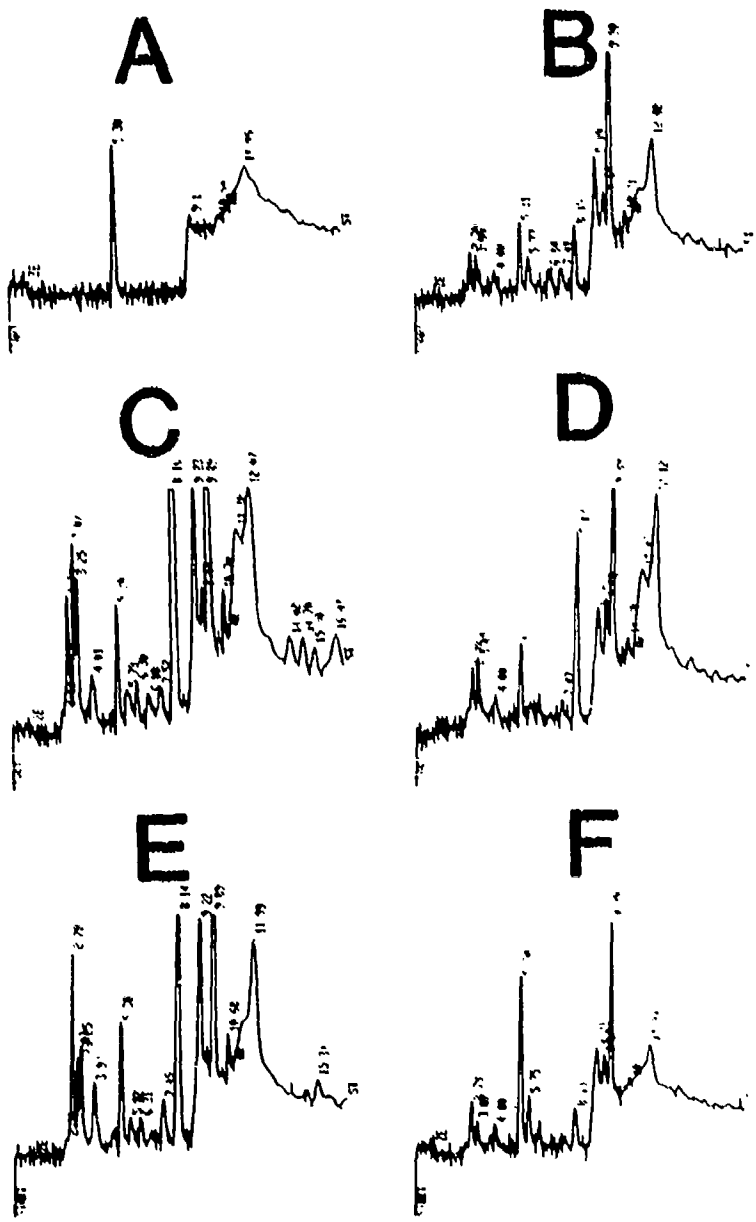


FIGURE 6. Comparison of HPLC chromatograms of patient samples with high fluorescence readings with neopterin standard. Five patient samples with high values for the 355 ex/420 em factor were chromatographed by HPLC with a fluorescence detector. Peaks are consistently present at 5.3 ± 0.1, 8.14 ± 0.2, 9.21 ± 0.2, and 9.9 ± 0.2 in each patient sample.

minutes under the conditions employed when these chromatograms were obtained. Three other fluorescent substances are consistently present at retention times at approximately 8.2, 9.2, and 9.9 minutes. The combination of the four peaks constitute an average of 72.1 percent of the fluorescence measured during the first 10 minutes of analysis by HPLC under these conditions.

DISCUSSION

We have separated four components that are consistently found in extracts of patient serum that have highly fluorescence p[illegible]chloric acid filtrates. These substances have fluorescent s[illegible]tral characteristics similar to nucleotide derivatives such as the pterins. Three of the components co-purify with and behave chromatographically similar to pterins, yet we have not established chemical identity with any of the commonly found pterin derivatives that we have compared them to.

It is difficult to determine accurately how much of the fluorescence seen in the original perchloric acid supernatant extracts from patient serum can be accounted for by these four chromatographically separated substances. Fluorescence is highly dependent on chemical structure as well as environmental factors such as pH. Since the pH of the detector chamber is approximately 7.4 and the fluorescent factors were originally measured in concentrated perchloric acid solution, it is not possible to quantitatively compare the fluorescence. We cannot assume that all of the fluorescent substances present in the supernatant extracts are detectable under the chromatographic conditions employed.

Several attempts have been made to derivatize the material and obtain a ionization fragmentation pattern by gas chromatography mass spectrometer, which is the most sensitive [illegible]thod of determination of chemical structure available. The suitable derivatives have not been attained to this point.

Further chemical characterization may not be possible on the small amount of material of limited purity that we have obtained so far. Future attempts at identification of these fluorescent substances will be attempted after collection and purification of milligram quantities of these substances.

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH COMPLETION REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS: Preliminary Studies of Lymphoid Subpopulations in a Burned Animal Model

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

David G. Burleson, PhD, Major, MS
Albert T. McManus, PhD
Ysidro Villareal
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS: Preliminary Studies of Lymphoid Subpopulations in a Burned Animal Model

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: David G. Burleson, PhD, Major, MS
Albert T. McManus, PhD
Ysidro Villareal
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

REPORT CONTROL SYMBOL: MEDDH-288(R1)

Whether defects in lymphocyte function contribute to inadequate response to infection in burn patients is not known. Surface receptors on lymphocytes are intimately involved in the execution and regulation of the immune response. It is possible that changes in the number of surface receptors of lymphocytes after burn injury could contribute to changes in the function of these cells. Lymphocyte surface markers on cells from control, burned, and burned-infected rats were quantified by flow cytometry after the binding of T-lymphocyte-specific monoclonal antibodies and fluorescent-labeled second antibody. Decreased fluorescence of the cells corresponded to decreased numbers of surface markers. The only surface antigen change seen in cells from burned but infected animals was on helper lymphocytes from blood. The number of surface antigens was reduced on these blood cells compared to unburned control. In burned-infected animals, the fluorescence intensity was decreased on all T-lymphocytes, helper lymphocytes, and suppressor lymphocytes from the lymph nodes. Spleen helper lymphocytes from burned-infected animals had increased fluorescence and thus an increased number of surface antigens compared to spleen cells from unburned controls. There was no correlation between cell size as measured by light scatter and the amount of binding of the fluorescent antibody. Thus, the changes in mean fluorescent intensity appeared to be due to increase or decrease in total receptor number rather than a change in receptor density.

PRELIMINARY STUDIES OF LYMPHOID SUBPOPULATIONS IN A BURNED ANIMAL MODEL

INTRODUCTION

The effect of burn injury on the *in vivo* function of lymphocytes is unclear. Many alterations of the reponse of burn patient lymphocytes to *in vitro* stimulation have been reported, but what changes in the lymphocyte response might be linked to suseptibility to opportunistic infection in these patients remains elusive. Lymphocyte function is clearly altered in burn patients, but the relationship of altered function to infection susceptibility is difficult to establish. Lymphocyte surface antigens are intimately related to immune function. Antibodies to the T-cell receptor (Ti or Leu4) stimulate mitosis in T-lymphocytes (1). Other surface receptors (used as subset markers) are necessarry for recognition of target cells, accessory cells, and antigen and the induction of interferons (2-3). Several activation antigens such as the interleukin-2 (IL2) receptor, transferrin receptor, and HLA-DR appear on the surface of mitogen-stimulated cells shortly after the cells have been expcsed to mitogens (4). Binding of IL2 by the IL2 receptor is required for continued activation of the lymphocytes (5). Antibodies to the IL2 receptor can modulate mitogenisis by interfering with IL2 binding (6). It is possible that the anomolies seen in burn patient lymphocyte response could be due to a change in the number of these surface receptors and thus the function and regulation of these cells.

---

[1]Meuer SC, Hodgdon JC, Hussey RE, *et al*: Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones. *J Exp Med* 158:988-993, 1983.

[2]Reinherz EL, Meuer S, Fitzgerald KA, *et al*: Antigen recognition by human T lymphocytes is linked to surface expression of the T3 molecular complex. *Cell* 30:735-743, 1982.

[3]Bhayani H and Falcoff R: T-Cell surface antigens defined by monoclonal antibodies involved in the induction of human interferon-gamma and interleukin 2. *Cell Immunol* 94:535-546, 1985.

[4]Burns GF, Battye FL, and Goldstein G: Surface antigen changes occurring in short-term cultures of activated human T lymphocytes: analysis by flow cytometry. *Cell Immunol* 71:12-26, 1982.

[5]Miller RA, Rozans MK, Ythier AA, *et al*: Stages of T cell activation: continued antigen dependence of IL 2-producing cells after IL 2 receptor expression. *J Immunol* 136:977-983, 1986.

[6]Lipkowitz S, Greene WC, Rubin AL, *et al*: Expression of receptors for interleukin 2: role in the commitment of T lymphocytes to proliferate. *J Immunol* 132:31-37, 1984.

We have measured the density of lymphocyte surface antigens from control, burned, and burned-infected rats to determine whether a relationship exists between the number of surface antigens present and infection susceptibility or the presence of infection.

MATERIALS AND METHODS

Male albino rats weighing 300 to 400 grams were randomly assigned to one of three groups, an unburned control group, a burned group, and a burned-infected group. All rats were anesthetized with pentobarbital (intraperitoneally, one milligram per 100 grams body weight), and those in the burned groups were shaved, placed in a plastic mold to accurately define the burn surface area, and subjected to a 30-percent total body surface area full-thickness burn by a 10-second immersion in boiling water. Infection was induced by placing one milliliter of a 16-hour broth culture containing approximately 10^8 Pseudomonas aeruginosa (Strain 59-1244) on the rat dorsum within one hour of scalding. Rats were sacrificed at 48 hours after infection and the blood, spleen, and mesenteric lymph nodes were taken for cell analysis. Rats were anesthetized with pentobarbital and exsanguinated by opening the body cavity and bleeding from the hepatic vein. A portion of the blood sample (one milliliter) from burned and burned-infected rats were cultured in trypticase soy broth to ascertain the presence of bacteria. Total leukocyte counts were made on a Coulter Counter and a blood smear for differential analysis was prepared. Spleen cells were obtained by disrupting the spleen and passing the cell suspension through a loosely packed cotton wool column to remove cell debris. Lymphoid cells from the blood and spleen were separated from other cells by centrifugation over a Ficoll-Hypaque density gradient. Lymph nodes were trimmed of excess tissue and minced with scissors over a 60-mesh stainless steel screen. The cells passing through the screen were passed through a loosely packed cotton wool column to screen out extraneous pieces of tissue. Lymph node cells obtained in this manner were greater than 95 percent lymphocytes and were used without further purification. Isolated cells from each tissue were washed and a portion of these cells were used to prepare a slide for differential analysis and the remaining cells were stained with anti-lymphocyte monoclonal antibodies obtained from Sera-Lab (Cambrige, United Kingdom). Monoclonal reagents attached to the cell surface were further bound with affinity purified, fluorescein-labeled goat anti-mouse immunoglobulin G (Fab2' fragments) as a second step reagent. The fluorescently stained cells were then analyzed by a fluorescence-activated cell sorter (Model 400, Becton Dickinson and Company). For each sample, 5,000 cells were analyzed and the number of cells fluorescently stained with anti T-lymphocyte (W3/13), suppressor/cytotoxic (OX-8), helper/inducer (W3/25), and Ia

antigen (OX-6) reagent was determined. A negative control using a monoclonal antibody to human T-cells was run with each cell preparation to determine the baseline mean autofluorescence. For measurements of mean fluorescence, nonlymphoid cell contamination was monitored by analyzing 90° scattered light. Those cells that fell outside the lymphocyte window were removed from analysis (gated).

RESULTS

The intensity of the fluoresecence from cells with fluoresecent-labeled monoclonal antibody bound their surface is proportional to the amount of the antibody bound. If we assume that the amount of antibody bound is proportional to the amount of antigen on the lymphocyte surface, linear fluorescent intensity of cells stained with monoclonal antibody would be proportional to the number of the surface antigens (7).

Fluoresent intensity was measured on blood, lymph node, and spleen lymphocytes from control, burned, and burned-infected rats on the second postburn day. The measurements from each group were compared pairwise using a t-test. Since log fluorescence measurements were more nearly normally distributed than the means of linear fluorescence, the means of log fluorescence measurements were compared rather than linear fluorescence. As shown in Table 1, the only significant differences between the mean fluorescent intensities between the cells from burned animals and those from controls was a slight decrease in mean fluorescence for helper lymphocytes from peripheral blood. However, there were several differences in the cells from burned-infected animals. Mean fluorescence intensities of the receptors for helper cells were reduced in blood and lymph nodes and increased in spleen. All of the T-cell markers were decreased in lymph nodes of burned-infected rats.

The change in fluorescence is due to changes in the absolute number receptors per cell. This absolute change in the number of receptors might occur by changing the number of receptors in the membrane without changing the total membrane surface area, i.e., a change in receptor density, or they could change via a change in the total membrane surface area, with a constant number of receptors per unit area of membrane. If the number of receptors per unit membrane surface area remains constant, then fluoresence intensity should correlate with the increase in membrane surface area.

---

[7]Crissman HA, Mullaney PF, and Steinkamp JA: Methods and applications of flow systems for analysis and sorting of mammalian cells. Methods Cell Biol 9:179-246, 1975.

TABLE 1

MEAN LOG FLUORESCENCE INTENSITY OF RAT LYMPHOID CELLS

Tissue	Antigen	Control Group	Burned Group	Burned-Infected Group
Blood	W3/13	136.4 ± 18.1	138.5 ± 17.8	132.3 ± 19.5
	W3/25	106.9 ± 20.4	101.5 ± 15.1	82.5 ± 12.9*
	OX-8	70.0 ± 14.4	68.8 ± 13.7	80.4 ± 19.4
	OX-6	59.4 ± 11.2	54.5 ± 10.4	54.6 ± 11.2
Spleen	W3/13	81.5 ± 12.0	86.7 ± 11.4	98.8 ± 18.6
	W3/25	69.9 ± 6.8	71.5 ± 7.6	85.1 ± 14.4*
	OX-8	53.9 ± 5.6	55.8 ± 10.4	64.5 ± 16.6
	OX-6	67.4 ± 13.5	67.1 ± 9.6	67.1 ± 17.1
Lymph Node	W3/13	126.4 ± 4.8	113.8 ± 22.7	105.2 ± 9.2**
	W3/25	106.9 ± 8.6	105.0 ± 8.3	93.4 ± 11.7*
	OX-8	80.1 ± 8.3	74.3 ± 10.9	70.3 ± 7.3*
	OX-6	85.5 ± 8.0	87.9 ± 8.5	87.5 ± 9.3

Log intensity is expressed in arbitrary fluorescence units standard deviation. The means for the three groups were compared pairwise using a t-test and the statistical significance of the P values determined using the Bonferroni adjustment. *0.05 level of significance compared to control group. **0.001 level of significance compared to control control.

Forward angle light scatter intensity was used to estimate approximate lymphocyte size. This light scatter is made up of several components, but the cross-sectonal area of the cell is the dominant contributor to the linear intensity (8). Cells of one type, i.e., lymphocytes compared to granulocytes or monocytes, have a fairly constant refractivity. When refractivities of the cells are equivalent, light scatter intensity provides a good measure of the relative size of the cells. The blood, spleen and lymph node lymphocytes from control, burned, and burned-infected animals were analyzed two days after injury. The mean light scatter intensity measurements obtained for cells from each tissue are shown in Table 2. The mean light scatter intensity from burned animals is not significantly different from control in any of the tissues examined. The scatter intensities do not vary greatly within each group. Although the blood lymphocytes from burned-infected animals showed a slight increase, the increased variability rendered the mean difference not significant.

The lack of change in forward scatter for any of the groups would indicate that fluorescent intensity changes were due to changes in receptor density rather than increased cell surface area. This was corroberated by a lack of correlation between fluorescent intensity and cell size. Cells from the blood, lymph nodes, and spleen from several animals in each group were analyzed to see if there was a correlation between light scatter (which is related to cell surface area) and the fluorescent intensity of each cell. The fluorecence and the scatter were compared on a cell-by-cell basis rather than on the mean values for 5,000 cells from the animals in each of the three groups. The fluorescence per cell varied much more than cell size. There was no significant correlation between cell size and fluorescent intensity for the cells from any of the tissues in any of the groups. We thus conclude that the changes in fluorescent intensity for each group of cells is due to changes in the density of the cell receptors and not to an increase in cell surface area at constant receptor density.

DISCUSSION

Peripheral blood lymphocytes from burned-infected rats contain a higher proportion of suppressor lymphocytes than lymphocytes from burned or control animals (9). The total

---

[8]Salzman GC: Light scattering analysis of cells. In Cell Analysis. Catsimpoolas N (ed). New York: Plenum Press, 1982, Volume 1, pp 111-143.

[9]Burleson DG, Vaughn GK, Mason AD Jr, et al: Flow cytometric measurement of rat lymphocyte subpopulations after burn injury and burn injury with infection. Arch Surg 122:216-220, 1987.

TABLE 2

MEAN SCATTER INTENSITY OF RAT LYMPHOID CELLS FROM CONTROL AND BURNED-INFECTED GROUPS (n = 10)

Tissue	Cell Type	Control Group	Burned Group	Burned-Infected Group
Blood	W3/13	100.0 ± 6.9	98.8 ± 8.7	106.8 ± 14.1
	W3/25	99.9 ± 6.2	98.5 ± 8.7	104.7 ± 15.4
	OX-8	100.0 ± 6.1	99.8 ± 9.9	105.8 ± 16.7
	OX-6	100.0 ± 6.0	100.2 ± 10.4	105.4 ± 15.7
Spleen	W3/13	100.0 ± 2.9	98.2 ± 4.2	99.0 ± 3.7
	W3/25	100.0 ± 2.9	98.8 ± 3.7	99.3 ± 3.5
	OX-8	100.0 ± 3.5	97.6 ± 5.5	98.3 ± 3.5
	OX-6	100.0 ± 2.8	99.6 ± 3.6	98.3 ± 2.3
Lymph Node	W3/13	100.0 ± 10.3	101.3 ± 6.2	96.4 ± 6.2
	W3/25	100.0 ± 10.4	101.4 ± 5.6	99.7 ± 7.2
	OX-8	100.0 ± 10.1	101.5 ± 5.9	98.6 ± 6.0
	OX-6	100.0 ± 10.7	101.6 ± 5.9	103.2 ± 14.1

Mean scatter intensity is shown as percent of mean scatter intensities for all controls on an experimental day.

number of lymphocytes is severely decreased in these animals, so the increased proportion of suppressor cells is actually caused by a selective depletion of helper cells. This increase in the proportion of suppressor cells appears in response to infection rather than contributing to infection susceptibility. Intuitively, one would assume that functional assessment of the cells would yield more valuable information on the ability of the immune system to resist infection than simply counting the cells in each lymphocyte subset. The relationship of cell surface receptor density to functionality is still unknown, but several interesting observations were made.

Increases in receptor density normally indicate increased responsiveness of the cells to environmental factors. If this is true for T-cell receptors in lymphocytes, there is a slight decrease in the responsiveness of helper cells in the blood of burned animals. There would be decreased responsiveness of T-lymphoid cells in burned-infected animals as well as decreased responsiveness of all lymph node T-cells and helper cells from the blood. The increased antigen on helper cells in the spleen is also interesting. We are now attempting studies to determine if function tests can be correlated to these surface marker changes.

PUBLICATIONS/PRESENTATIONS

Burleson DG: Use of light scatter in flow cytometry measurements of rat lymphocyte subpopulations after burn injury and burn injury with infection. Presented at the Sixth Annual Meeting of the Surgical Infection Society, Chicago, Illinois, 21-22 April 1986.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS: Therapy with IgG and T_4 in Burn Patients

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Anne E. Missavage, MD, Major, MC
Khan Z. Shirani, MD, Lieutenant Colonel, MC
George M. Vaughan, MD, Lieutenant Colonel, MC
Albert T. McManus, PhD
David G. Burleson, PhD, Major, MS
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS: Therapy with IgG and T_4 in Burn Patients

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: Anne E. Missavage, MD, Major, MC
Khan Z. Shirani, MD, Lieutenant Colonel, MC
George M. Vaughan, MD, Lieutenant Colonel, MC
Albert T. McManus, PhD
David G. Burleson, PhD, Major, MS
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

Infection is the major cause of death after burns. The aim of this randomized study is to determine whether or not IgG and T_4 replacement therapy in burned patients will alter the frequency or severity of septic complications. Within five days postburn, patients age 18 or older with a 20 to 80 percent probability of mortality are randomized to receive either no immunoglobulin G or immunoglobulin G (IGIV, Cutter Biological) in a dose of 500 milligrams per kilogram preburn weight twice weekly for a four-week or longer period until they are completely healed. Among the immunoglobuin G recipients and controls, those with a free thyroxine index less than four during the second postburn week are further randomized to thyroxine or no thyroxine replacement groups.

During administration of intravenous gamma globulin, all patients are closely monitored for untoward effects of the drug, and thus far with this agent, no side effects have been seen. On all patients, routine laboratory tests are performed prior to, during, and after completion of the study. Study patients are followed clinically for development of infection and, on a regular basis, urine and sputum specimens are cultured for surveillance. Blood cultures and burn wound biopsies are obtained when clinically indicated. In these subjects, serum immunoglobuin G is measured with radial immunodiffusion and by nephelometry, leukocyte function by flow cytometry, T-lymphocyte function by tritiated thymidine incorporation, subset analysis of T-lymphocytes by monoclonal antibodies, and antibody-producing capability of B-cells by the

assay of immunoglobulins harvestable from supernatants of cultured lymphocytes.

Thus far, 61 patients have been entered into this study; 31 have received immunoglobulin G and 30 have served as controls. Two subjects with a low free thyroxine index were given thyroxine replacement. This ongoing study will be concluded when approximately 100 patients have been enrolled. Data will then be analyzed to assess the efficacy of this form of therapy for prevention of infection in burned patients.

THERAPY WITH IgG AND T_4 IN BURN PATIENTS

INTRODUCTION

In thermally injured subjects, relentless sepsis remains a major problem despite the availability of potent antibiotics and major advances in the areas of fluid therapy, nutritional support, and burn wound management. Profound suppression of humoral and cellular immune responsiveness and of neutrophil function represent characteristic defects in the defense mechanisms of burn patients. This state of acquired immune deficiency in burn patients is somewhat akin to the depressed immune status of patients with primary immune deficiency syndromes and manifests itself as deficiency in the cell-mediated and humoral components of immunity; patients in both groups are susceptible to infection (1,2). For two decades, immunoglobulin G (IgG) replacement in patients with immune deficiency syndromes has been recognized as the mainstay of prophylaxis and has distinctly reduced the incidence of infection in these individuals. In burn patients, the lack of a protective skin barrier and the use of invasive monitoring enhance the risk of colonization of deeper tissues with a wide variety of organisms. Associated defects in the immune defenses may further aid the evolution of infectious processes. This study proposes immunomodulation of burned patients by IgG replacement during the first four weeks postburn when concentrations of endogenous IgG in these subjects are subnormal and they are prone to systemic infection. Burn-induced alterations in immunologic, biochemical, and hormonal indices are being monitored and will be compared with the modifications produced in those indices subsequent to therapy with IgG and thyroxine (T_4).

Burn patients, like those with other nonthyroidal illnesses, often develop low serum concentrations of thyroid hormone (3). In patients with other nonthyroidal illnesses,

---

[1]Arturson G, Hogman CF, Johansson SGO, and Killander J: Changes in immunoglobulin levels in severely burned patients. Lancet 1:546-548, 1969.

[2]Buckley RH: Immunoglobulin replacement therapy: indications and contraindications for use and variable IgG levels achieved. Alving BM (ed). In Immunoglobulins: Characteristics and Uses of Intravenous Preparations. Washington, DC: United States Government Printing Office, 1980, pp 3-8.

[3]Becker RA, Vaughan GM, Ziegler MG, Seraile LG, Goldfarb IW, Mansour EH, McManus WF, Pruitt BA Jr, and Mason AD Jr: Hypermetabolic low triiodothyronine syndrome of burn injury. Crit Care Med 10:870-875, 1982.

the development of low serum T_4 concentration is associated with greater risk of mortality (4,5). We have also found that in burn patients, nonsurvivors tend toward low T_4 and mental obtundation. Both these indices are correlated with mortality (6). The obvious question is whether replacement of thyroid hormone will repair mental status and/or prevent mortality. Septic processes may underly or contribute to obtundation and mortality. Tracer T_4 accumulates at sites of infection. Phagocytotic function of granulocytes appears related to thyroid hormones and appears to be suppressed in classical hypothyroidism as well as in critically ill burn patients. It is reasonable to hypothesize that T_4 replacement therapy may improve neutrophil function and enhance opsonization of microorganisms, making them susceptible to phagocytosis by neutrophils.

In burn patients, triiodothyronine (T_3), the peripherally-derived, more active product of T_4, is lowered more in serum than is T_4, but reduced T_3 is not a good indicator of mortality since survivors also usually have suppressed T_3. For uptake, the brain prefers circulating T_4 over T3 (7) and it is not yet known what preference for uptake is exhibited by the inflammatory and immune systems. A trial of T_3 replacement therapy showed no effect on mortality in burns (3). However, many of the patients in that study did not have suppression of serum T_4 (as judged by data in placebo patients). In that study, the negative feedback due to exogenous T_3 suppressed serum T_4, obscuring the interpretation of T_4 values and minimizing any beneficial effect of endogenous T_4, should there be one separate from the effect of T_3. Finally, that study was undertaken without any attempt to repair the serum IgG status. Whether IgG therapy has been instituted or not in a given patient, if free thyroxine index (FT_4I) remains below four in the second postburn week, the patient is randomized to therapy with T_4 or to no T_4 therapy. For reasons already mentioned, a combined IgG and T_4

---

[4]Slag MF, Morley JE, Elson MK, Crowson TW, Nuttall FQ, and Shafer RB: Hypothyroxinemia in critically ill patients as a predictor of high mortality. JAMA 245:43-45, 1981.

[5]Kaptein EM, Weiner JM, Robinson WJ, Wheeler WS, and Nicoloff: Relationship of altered thyroid hormone indices to survival in nonthyroidal illnesses. Clin Endocrinol 16:565-574, 1982.

[6]Vaughan GM, Mason AD Jr, McManus WF, and Pruitt BA Jr: Alterations of Mental Status and Thyroid Hormones after Thermal Injury. J Clin Endocrinol and Metab 60:1221-1225.

[7]Obregon MJ, Roelfsema F, Morreale de Escobar G, Escobar del Rey F, and Querido A: Exchange of triiodothyronine derived from thyroxine with circulating triiodothyronine as studied in the rat. Clin Endocrinol 10:305-315, 1979.

replacement study to enhance both antibody levels and leukocyte function has been undertaken to determine if their possible beneficial effects are permissive, additive, or potentiative. The effects of IgG and T_4 will be assessed by measuring indices of humoral and cellular immunity, incidence of sepsis, changes in mental status, thyroid and other hormones in the serum, and mortality.

MATERIALS AND METHODS

This unblinded, randomized study evaluates the efficacy of exogenously administered IgG and T_4 for prophylaxis of infection in burn patients. Adult patients are offered admission to the study if, for that particular patient's age, the total burn size is such that the probability of death is between 20 and 80 percent, based on previous logistic regressions over a large population of burn patients receiving about the same general care as our patients are expected to receive.

Inclusion Criteria. Burn patients of either sex more than 18 years of age with a probability of mortality of 20 to 80 percent admitted to this Institute during the first five days postburn are considered eligible for this study.

Exclusion Critiera. Patients under the age of 18, females of childbearing age with a positive pregnancy test, patients with less than a 20 percent or greater than an 80 percent chance of mortality (based on age and total burn size), and those admitted to this Institute more than five days postburn are not eligible for this study.

Design. Each patient is randomized into the IgG treatment group (those who receive IgG) or the IgG control group (those who do not receive IgG). Those patients who develop a low serum T_4 and FT_4I below four during the second week postburn are randomized to T_4 replacement or no T_4 replacement therapy. IgG therapy (500 mg/kg IV infused over four hours twice weekly for two weeks) begins between postburn days two and five. The dose of T_4, 0.2 milligrams per day, is adjusted to maintain midnormal serum T_4, continuing, if necessary, until recovery. IgG administration is advanced to the immediate postoperative period for patients undergoing surgery.

Hormonal, Biochemical, and Immunological Monitoring. From the time of initial randomization, blood samples are obtained twice weekly (just before the IgG infusion in the group receiving IgG) for hormonal, biochemical, and immunological monitoring. Sometimes, additional samples for thyroid hormone analysis are necessary to adjust the dosage of T_4. Indices of cellular and humoral immunity, bacteriologic and virologic surveillance, and hormonal responses are examined twice weekly

over a four-week period. However, patients with persistently abnormal findings often require further investigation beyond four weeks.

Cellular and humoral immunity. Indices of immune responsiveness include:

1. Measurements of T-cells and their subsets and B-cells and their subsets by flow cytometry using the immunofluorescence technique.

2. Polyclonal lymphocyte activation by standard mitogens.

3. Measurements of total and differential leukocyte count as well as chemiluminescence.

Bacteriologic and virologic surveillance. Surveillance for infectious agents includes bacteriologic (blood, urine, and sputum cultures) and virologic (urine and throat washings for CMV culture and CMV antibody titers) tests. Blood cultures are drawn only when clinically indicated.

Hormonal reponses. Hormonal response testing includes catechols, steroids, renin-angiotensin-aldosterone, vasopressin, and thyroid hormones (T_4, T_3, rT_3, TSH, T_3U).

Immunoglobulins are analyzed by nephelometry (8). A fluorescence-activated cell sorter is employed to separate various lymphocyte subpopulations (9) and monoclonal cells (10). Chemolinescence is measured as previously described (11). Bacteriologic and viral isolation is performed by standard methods. Hormones are measured by RIA with standard kits (thyroid hormones, cortisol, aldosterone, PRA) and by procedures developed at this Institute (angiotensin I and II).

---

[8]Killingsworth LM and Savory J: Manual nephelometric methods for immunochemical determination of immunoglobulin IgG, IgA, and IgM in human serum. Clin Chem 18:335-339, 1972.

[9]Herzenberg LA and Herzenberg LA: Analysis and separation using the fluorescence activated cell sorter (FACS). Weir DM (ed). In Handbook of Experimental Immunology. Oxford: Blackwell Scientific Publications, 1978, pp 22.1-22.21.

[10]Goldstein G, Lifter J, and Mittler R: Immunoregulatory changes in human disease detected by monoclonal antibodies to T lymphocytes. McMichael AJ (ed). In Clinical Medicine. London: Academic Press, 1982, pp 39-70.

[11]Allen RC and Pruitt BA Jr: Humoral-phagocyte axis of immune defense in burn patients. Chemoluminigenic probing. Arch Surg 117-133-140, 1982.

RESULTS

See Tables 1 and 2 for results as of 30 September 1985.

DISCUSSION

Overwhelming sepsis significantly contributes to the mortality of patients with extensive burns (12). A multitude of pertubations encountered in the immunologic defenses of burn patients point to the immunosuppressive nature of burn injury. Burn-induced changes in the humoral and cellular responses include hypogammaglobulinemia (13), reduction in the complement levels (14), depressed serum opsonic activity (15), decreased granulocyte chemotaxis (16), anergy to recall antigens (17), prolonged survival of skin allograft (18), depressed response in autologous mixed lymphocyte culture, and decreased OKT_4/OKT_8 ratio (19). In the past, encouraged by the favorable experience observed in patients with immune deficiency syndromes (2), two clinical trials in burn patients (20,21) employed IgG for prophylaxis of infection with apparently contradictory results. IgG administered in sufficient amounts

---

[12]Sevitt S: A review of the complications of burns, their origin and importance for illness and death. J Trauma 19:358-369, 1979.

[13]Munster AM, Hoagland HC, and Pruitt BA Jr: The effect of thermal injury on serum immunoglobulins. Ann Surg 172:965-969, 1970.

[14]Fjellstrom KE and Arturson G: Changes in the human complement system following burn trauma. Acta Path Microbiol Scand 59:257-270, 1963.

[15]Bjornson AB and Alexander JW: Alterations of serum opsonins in patients with severe thermal injury. J Lab Clin Med 83:372-382, 1974.

[16]Warden GD, Mason AD Jr, and Pruitt BA Jr: Evaluation of leukocyte chemotaxis in vitro in thermally injured patients. J Clin Invest 54:1001-1004, 1974.

[17]Casson P, Solowey AC, Converse JM, et al: Delayed hypersensitivity status of burned patients. Surg Forum 17:268-270, 1966.

[18]Ninnemann JL, Fisher JC, and Frank HA: Prolonged survival of human skin allografts following thermal injury. Transplantation 25:69-72, 1978.

[19]Antonacci AC, Calvano SE, Reaves LE, Welte K, Mertelsmann R, and Shires GT: Restoration of autologous mixed lymphocyte responses in burn patients following in vitro addition of interleukin-2: analysis of responder cell populations with monoclonal antibodies. Surg Forum XXXIV:108-110, 1983.

[20]Kle: ides NA, Arana JA, Bazan A, et al: Role of infection in mortality from severe burns. New Engl J Med 267:317-323, 1962.

TABLE 1. General Information

	TREATMENT GROUP	CONTROL GROUP
Number of Patients	31	30
Age (Mean)	44.0	44.7
Extent of Burn (Mean)	42.9	46.4
Patients with Infection	22	24
Survivors	18	19
Nonsurvivors	11	9

in the early postburn period was credited with improved survival in children (20). However, in adult patients, IgG therapy in relatively small doses administered during a variable postburn period failed to show any beneficial effects (21). During the past, clinical trial of IgG in burn patients has been impeded on two counts, by nonexistence of suitable IgG preparation for intravenous use and by a lack of information about the kinetics of infused IgG in burn patients. Currently available IgG preparations are completely devoid of the vasomotor and other side effects reported with older products. This has been achieved by the modification of IgG through a process of reduction and alkylation (Gammimune, Cutter Biological) and by reduction alone (IGIV, pH 4.25, Cutter Biological). This latter preparation, however, preserves more than 90 percent of the IgG molecule in its native monomer form. In recent kinetic studies, we have demonstrated that twice weekly IgG infusions given in a dose of 500 mg/kg are sufficient to normalize serum IgG concentration in burn patients (22). Within 48 hours of thermal injury, serum IgG is at its lowest concentration and thereafter, gradually returns to normal over a three to four-week period (12). Based on the state of current knowledge, it is reasonable to test whether immunomodulation of burn victims with IgG will aid in reduction of infection and thus improve survival in these immunocompromised individuals. For therapeutic interventions

---

[21]Stone HH, Graber CD, Martin JD Jr, and Kolb L: Evaluation of gammaglobulin for prophylaxis against burn sepsis. Surgery 58:810, 1965.

[22]Shirani KZ, Vaughan GM, McManus AT, Amy BW, McManus WF, Pruitt BA Jr, and Mason AD Jr: Replacement therapy with modified immunoglobulin G in burn patients: preliminary kinetic studies. Am J Med 76:175-180, 1984.

TABLE 2. Number of Infections

	TREATMENT GROUP		CONTROL GROUP	
	Number of Patients	Number of Infections	Number of Patients	Number of Infections
Pneumonia	17	26	19	29
Bacteremia	13	20	12	20
Burn Wound Invasion	2	3	2	2
Urinary Tract Infection	6	7	6	11
Cellulitis	7	7	7	7
Other	1	1	4	5
TOTAL NUMBER OF INFECTIONS		64		74

aimed at prophylaxis, it is imperative that such therapy be instituted during the period of extreme vulnerability which preceeds clinical manisfestations of established sepsis. For this reason, patients in this study receive IgG therapy during the early postresuscitation period when concentrations of their endogenous IgG are subnormal. This study attempts to characterize modifications produced in burn-induced immunologic and endocrine responses and their correlation with subsequent development of sepsis in the study population.

Despite the widely known elevation of total metabolic rate following burns, oxygen consumption and bactericidal activity are suppressed in granulocytes of severely burned patients (23). Leukocyte oxygen consumption is low in nonburned, but classically hypothyroid, patients and increases with thyroid hormone therapy (24). A review (25) of the granulocyte cellular metabolic burst that accompanies phagoctosis points out that this process includes oxygen utilization, degradation of thyroid hormones, and iodination of protein and suggests that the hormones may contribute to the microbicidal activity of the cell. Labelled T_4 is concentrated in areas of acute infection in humans. We have, therefore, pointed out that burn patients with deficient thyroid hormone may be functionally hypothyroid in regard to host defense (26). Since immunoglobulins may promote defense partly by attaching to microorganisms, enabling their phagoctyosis by granulcoytes, we are attempting to restore IgG concentrations, and also attempting to improve a defect in granulocyte function by replacing T_4 in those patients with deficient T_4. At present, because the study is incomplete (see Table 1), no conclusions from these data have been reached. A detailed analysis of results will follow at the completion of the study.

---

[23]Canonico PG, McManus AT, and Powanda MC: Biochemistry and function of the neutrophil in infected and traumatized hosts. Dingle JT, Jacques PJ, and Shaw IH (eds). In Lysosomes in Applied Biology and Therapeutics. Amsterdam: North-Holland Publishing Company, 1979, pp 287-326.

[24]Bisset SK and Alexander WD: The effect of intravenous injections of triiodothyroacetic acid and l-triiodothyronine on the oxygen consumption of circulating human leucocytes. Quart J Exp Physiol 46:50-53, 1961.

[25]Klebanoff SJ and Clark RA: The metabolic burst. In The Neutrophil: Function and Clinical Disorders. Amsterdam: North-Holland Publishing Company, 1978, pp 283-408.

[26]Becker RA, Vaughan GM, Goodwin CW Jr, Ziegler MG, Zitzka CA, Mason AD Jr, and Pruitt BA Jr: Interactions of thyroid hormones and catecholamines in severely burned patients. Rev Inf Dis 5(S5):S908-S913, 1983.

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DAOG1842	85 10 01	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
85 10 01	D	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BD	304
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY87-01		

11. TITLE *(Precede with Security Classification Code)*
(U) Role of Thyroid Hormones in Burn Pathophysiology

12. SUBJECT AREAS
06 05 Clinical Medicine 06 01 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
79 08	CONT	DA	C

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED APPROVED BY
a. DATE EFFECTIVE
b. CONTRACT/GRANT NUMBER
c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
86	1.2	70
87	1.2	78

19. RESPONSIBLE DOD ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720

20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF PRINCIPAL INVESTIGATOR: VAUGHAN, G M
d. TELEPHONE NUMBER *(Include area code)*: 512-221-5416
e. NAME OF ASSOCIATE INVESTIGATOR *(If available)*
g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

21. GENERAL USE: FINA
MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) L-Triiodothyronine; (U) Therapy; (U) Deiodinase; (U) Hypothyroidism; (U) Thyroxine; (U) Volunteers;

23 TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

22. (Continued) (U) Lab Animals: (U) Rats; (U) Hamsters; (U) RAII

23. (U) To assess the abnormalities of thyroid function in burn patients.

24. (U) To characterize thyroid dysfunction with serum thyroid-stimulating hormone measurement and altered thyroxine binding with thyroxine-binding globulin measurement in burn patients.

25. (U) 8510 - 8609. We first determined that a new highly specific and sensitive magnetic immunoradiometric assay of thyrotrophin (TSH) could indeed assess depression of serum TSH. All 26 nonburn patients from whom samples could be obtained with thyrotoxicosis had TSH values lower than the lowest TSH of 16 normal control subjects. Another set of 10 normal control subjects were compared with seven burn survivors and five nonsurvivors sampled twice weekly. Even in the first two weeks (before dopamine was used in the nonsurvivors), thryoxine (T4), dialyzable free thyroxine (FT4), and TSH rose with time in survivors and fell markedly in nonsurvivors with a positive overall correlation between FT4 and TSH. Mean T4 and FT4 was low initially in survivors and nonsurvivors, whereas TSH was initially in the normal range. Whereas the T4 dialyzable fraction was elevated in the patients (versus controls), thyronine-binding globulin (TBG) was not low, and a calculated predicted T4 dialyzable fraction (based on the T4, TBG, globulin, and mass equation) was not elevated. Thus, initally low FT4 even in survivors was not associated with elevated TSH, suggesting an early

DD FORM 63 MAR 1498 EDITION OF MAR 68 IS OBSOLETE

CONTINUATION OF DD FORM 1498 FOR "ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY"

deficient secretion of TSH. Whereas this corrected with time in survivors (some with transiently elevated TSH), deficient levels of TSH worsened with time in nonsurvivors along with a plunge of total and FT4 and triiodothyronine to very low levels, suggesting a markedly different response of central control of the thyroid axis in nonsurvivors. The T4 binding deficit in serum is not due to a deficiency of TBG in burn patients and thus may be related to the presence of a binding inhibitor or abnormalities of other binding proteins.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY: Thyroid Hormone Economy After Burns - Two Patterns of Thyrotropin Alteration and Lack of Change in Thyronine Binding Globulin

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

George M. Vaughan, MD, Lieutenant Colonel, MC
Richard A. Becker, MD*
Khan Z. Shirani, MD, Lieutenant Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

*Division of Endocrinology
Department of Medicine
University of Texas Health Science Center
San Antonio, Texas

ABSTRACT

PROJECT NUMBER: 3M161102BS10-00, BASIC RESEARCH

PROJECT TITLE: ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY: Thyroid Hormone Economy After Burns - Two Patterns of Thyrotropin Alteration and Lack of Change in Thyronine Binding Globulin

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: George M. Vaughan, MD, Lieutenant Colonel, MC
Richard A. Becker, MD
Khan Z. Shirani, MD, Lieutenant Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

We report a prospective study of serum thyroxine, triiodothyronine, their dialyzable fractions and free concentrations; thyronine binding globulin by radioimmunoassay; and thyrotropin measured by a sensitive and specific immunoradiometric assay in seven burn survivors, five burn nonsurvivors, and ten control subjects. With exception of dopamine given after the second postburn week in nonsurvivors, drugs known to affect the thyroidal axis were avoided. On the basis of the mass equation and measured thyroxine and thyronine binding globulin, an increase in the thyroxine dialyzable fraction was not predicted; however, measured thyroxine dialyzable fraction was increased in burns in association with normal thyronine binding globulin concentration. A decrease in mean free thyroxine was observed in the first postburn week, free triiodothyronine was profoundly depressed, and thyrotropin was normal. Thereafter, survivors and nonsurvivors differed markedly. Thyroxine, free thyroxine, and thyrotropin rose over the first two weeks in survivors and fell in nonsurvivors; the mean slopes differed significantly prior to dopamine infusion in nonsurvivors. Total and free thyroxine and triiodothyronine returned toward normal in survivors, with thyrotropin normal to elevated. These data imply an abnormality such as decreased thyroidal secretion or accelerated thyroxine disposal and altered generation or disposal of triiodothyronine occurring at a level below the pituitary. The thyrotrophes eventually exhibit a qualitatively normal response in survivors, but fail in nonsurvivors beginning very early in the clinical course. Given the degree of supression of thyroxine and

triiodothyronine in survivors, failure of thyrothropin to increase earlier in the clinical course may represent a relative inadequacy of thyrothropin secretion, but falling thyrothropin is a harbinger of fatal outcome and suggests hypothalamic or pituitary hypothyroidism. A defect is observed in serum thyroid hormone binding in both survivors and nonsurvivors which is not due to deficient thyronine binding globulin and does not prevent depression of free thyroxine and free triiodothyronine.

BURNS
DIALYZABLE FRACTION
THYRONINE
THYRONINE BINDING GLOBULIN
THYROTROPIN
TRIIODOTHYRONINE

THYROID HORMONE ECONOMY AFTER BURNS: TWO PATTERNS OF THYROTROPIN ALTERATION AND LACK OF CHANGE IN THYRONINE BINDING GLOBULIN

INTRODUCTION

Thyroid function in burn injury has been studied (1-14), and these reports indicate reduced circulating concentrations of thyroxine (T4) and triiodothyronine (T3) in burned humans, rats, and Syrian hamsters. The role of thyrotropin (TSH) in

---

[1]Cope O, Nardi GL, Quijano M, et al: Metabolic rate and thyroid function following acute thermal trauma in man. Ann Surg 137:165-174, 1953.

[2]Becker RA, Wilmore DW, Goodwin CW Jr, et al: Free T4, free T3, and reverse T3 in critically ill, thermally injured patients. J Trauma 20:713-721, 1980.

[3]Becker RA, Vaughan GM, Goodwin CW Jr, et al: Plasma norepinephrine, epinephrine, and thyroid hormone interactions in severely burned patients. Arch Surg 115:439-443, 1980.

[4]Becker RA, Goodwin CW, Mason AD Jr, et al: Splanchnic exchange of free thyroid hormones in critically ill burn patients. In Thyroid Research VIII. Stockigt JR and Nagataki S (eds). Sydney: Pergamon Press, 1980, pp 465-468.

[5]Kien CL, Vanjonack WJ, and Bode HH: Low serum reverse T3 concentration in burned children: its relationship to nutritional state. Am J Clin Nutr 33:1215-1219, 1980.

[6]Smeds S, Kagedal B, Lieden G, et al: Thyroid function after thermal trauma. Scand J Plast Reconstr Surg 15:141-148, 1981.

[7]Becker RA, Vaughan GM, Ziegler MG, et al: Hypermetabolic low triiodothyronine syndrome of burn injury. Crit Care Med 10:870-875, 1982.

[8]Dolecek R: Burn stress and its endocrine consequences. A review. Acta Chir Plast 26:107-128, 1984.

[9]Calvano SE, Chiao J, Reaves LE, et al: Changes in free and total levels of plasma cortisol and thyroxine following thermal injury in man. JBCR 5:143-151, 1984.

[10]Vaughan GM, Mason AD Jr, McManus WF, et al: Alterations of mental status and thyroid hormones after thermal injury. J Clin Endocrinol Metab 60:1221-1225, 1985.

[11]Shirani KZ, Vaughan GM, Pruitt BA Jr, et al: Reduced serum T4 and T3 and their altered serum binding after burn injury in rats. J Trauma 25:953-958, 1985.

[12]Vaughan GM, Shirani KZ, Vaughan MK, et al: Hormonal changes in burned hamsters. Endocrinology 117:1090-1095, 1985.

[13]Scott DE, Vaughan GM, and Pruitt BA Jr: Hypothalamic neuroendocrine correlates of cutaneous burn injury in the rat: I. Scanning electron microscopy. Brain Res Bull 17:367-378, 1986.

the altered thyroid function following burn injury is not fully understood. The elevated dialyzable fractions of T4 and T3 (T4DF, T3DF) indicate a serum thyronine binding defect in burn injury (10-12), though whether it results from reduced concentrations of thyronine binding globulin (TBG) has not been clarified. The purposes of this study were to assess the course of serum thyroid axis hormones, including TSH, in burn injury and to assess serum T4 binding in the light of TBG measurements.

MATERIALS AND METHODS

Preliminary Observations. Serum samples from 26 nonburned endocrine clinic patients were identified as thyrotoxic by elevation of the free T4 index (FT4I), as compared with samples from 16 normal adults (Figure 1). In order to document whether the TSH assay could detect a reduction in TSH concentration below the normal range, should such a reduction occur, TSH was assessed in both groups of samples.

Protocol. Serum samples from burn patients (men except for one woman among the survivors) were obtained two to three times per week in the morning for assay of substances related to the thyroid axis. Patients received standard postburn care, including fluids and electrolytes for initial resuscitation and subsequent evaporative water loss, alternating topical mafenide acetate and silver sulfadiazine in the open treatment of wounds, excision and grafting when indicated, vigorous nutritional support (2), systemic antibiotics if needed for infection, parenteral morphine as needed for pain, and oral antacids to prevent Curling's ulcers. Treatment did not include topical or systemic agents containing iodine, carbohydrate-active steroids, or heparin. Dopamine infusions were employed in nonsurvivors as needed only after the second week postburn, and samples taken after addition of other sympathomimetic amines for cardiovascular support were excluded. Deaths occurred 12 to 37 days after injury, and analyzed samples were collected up to 6 to 19 days before death. Samples from 10 healthy subjects (including one woman not receiving estrogen) were used as controls. The charactistics of the patients and controls are given in Table 1.

Assays. Radioimmunoassays of thyroxine and triiodothyronine and charcoal *in vitro* T3 uptake (T3U) were performed with kits from Diagnostic Products, Los Angeles,

---

[14]Vaughan GM, Pruitt BA Jr, Shirani KZ, *et al*: The thyroid axis and brain 5'-monodeiodination of thyroxine in the burned rat model of nonthyroidal illness. Neuroendocrine Let (in press).

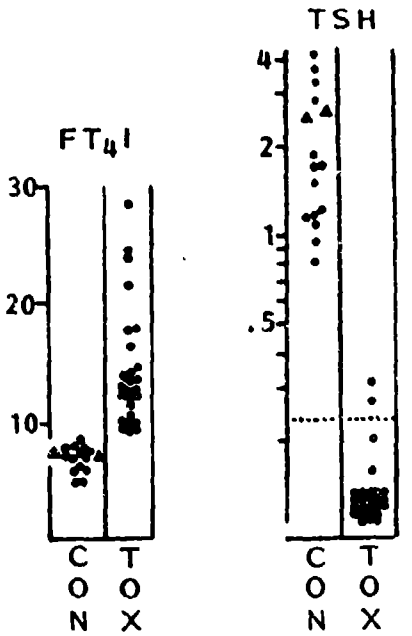


FIGURE 1. Free thyroxine index (FT4I) and thyrotropin (TSH, μU/ml) from a preliminary study in normal control (CON) subjects including two women (triangles) on exogenous estrogen and in patients with thyrotoxicosis (TOX) defined as FT4I above the normal limits. The dotted line is the least detectable TSH.

California. Reverse T3 (rT3) was determined with radioimmunoassay kits from Serono Labs, Braintree, Massachusetts. The dialyzable fractions at equilibrium (T4DF and T3DF) were determined at the Nichols Institute, San Juan Capistrano, California, as were TBG and thyroglobulin by radioimmunoassay. Least detectable concentrations were: T4, 0.2 μg/dl; T3, 7 ng/dl; rT3, 2 ng/dl; TBG, 0.016 mg/dl; and thyroglobulin, 1 ng/ml. Free concentrations (FT4, FT3) were calculated as the respective product of the T4 or T3 and the T4DF or T3DF. FT4I was the product of the T4 and T3U divided by the normal reference sample T3U provided in the kit.

TABLE 1

CHARACTERISTICS OF THE PATIENTS

	CONTROLS (n = 10)			BURN SURVIVORS (n = 7)			BURN NONSURVIVORS (n = 5)		
	Mean	Standard Error	Range	Mean	Standard Error	Range	Mean	Standard Error	Range
TBS (%)	-	-	-	46.8	3.0	38-62	47.8	9.3	21-70
FTB (%)	-	-	-	27.7	5.5	0-42	5.8	2.1	0-11
Age (Years)	36.4	3.84	26-57	33.7	5.7	21-64	50.2	9.0	33-84

NOTE: TBS = total burn size as percentage of the total body surface area, FTB = full-thickness (third degree) burn size as percentage of the total body surface area.

TSH was measured in the triple monoclonal antibody immunoradiometric assay with magnetic separation (15-16) provided as kits from Serono Labs. In nine assays, each containing nine replicates of zero-standard and duplicates of each of three external control samples, the mean least detectable limit (from the mean zero-standard counts plus 2 standard deviation) was 0.22 μU/ml, and the interassay coefficient of variation was 9.7, 8.8, and 6.5 percent for 1.5, 3.8, and 4.2 μU/ml, respectively. This agreed well with the detection limit (0.2 μU/ml) and interassay variation (7.4 and 4.5 percent at 1.6 and 4.4 μU/ml) previously reported for this method (16).

Analysis. Statistical tests were performed on a VAX 11/780 computer using the UCLA BMDP program (17). Within-patient means for samples in a given postburn week were used as data (Figures 2 and 3, Table 2), and comparisons of between-patient means were made by t-tests with the Bonferroni correction for multiplicity of comparisons. Analyses of covariance on individual data were used to compare survivors with nonsurvivors with respect to the correlation of T4 variables and TSH (as dependents) with time postburn and of T4 variables (dependents) with TSH in samples from the first two weeks before nonsurvivors received dopamine. In these analyses (Table 3), hormone values for a given patient were divided by the respective mean of the values used in order to minimize between-patient variability. A predicted T4DF was calculated on the basis of the molar concentrations of T4 and TBG, their association constant k (1.7×10^{10} 1/M), and the mass action law for a ligand to protein molecular ratio of 1:1 (18-20).

---

[15]Rattle SJ, Purnell DR, Williams PI, et al: New separation method for monoclonal immunoradiometric assays and its application to assays for thyrotropin and human choriogonadothropin. Clin Chem 30:1457-1461, 1984.

[16]Cobb WE, Lamberton RP, and Jackson IM: Use of a rapid, sensitive immunoradiometric assay for thyrotropin to distinguish normal from hyperthyroid subjects. Clin Chem 30:1558-1560, 1984.

[17]Dixon WJ (ed). BMDP Statistical Software. Berkeley: University of California Press, 1983.

[18]DeGroot LJ and Stanbury JB: Hormone synthesis, secretion, and action. In The Thyroid and Its Diseases. New York: John Wiley and Sons, 1975, pp 37-109.

[19]Pearlman WH: Measurement of testosterone binding sites. Acta Endocr (Kobenhaun) 147:225+, 1970.

[20]Marshall JS and Pensky J: Studies on thyroxine-binding globulin (TBG). III. Some physical characteristics of TBG and its interaction with thyroxine. Arch Biochem Biophysics 146:76-83, 1971.

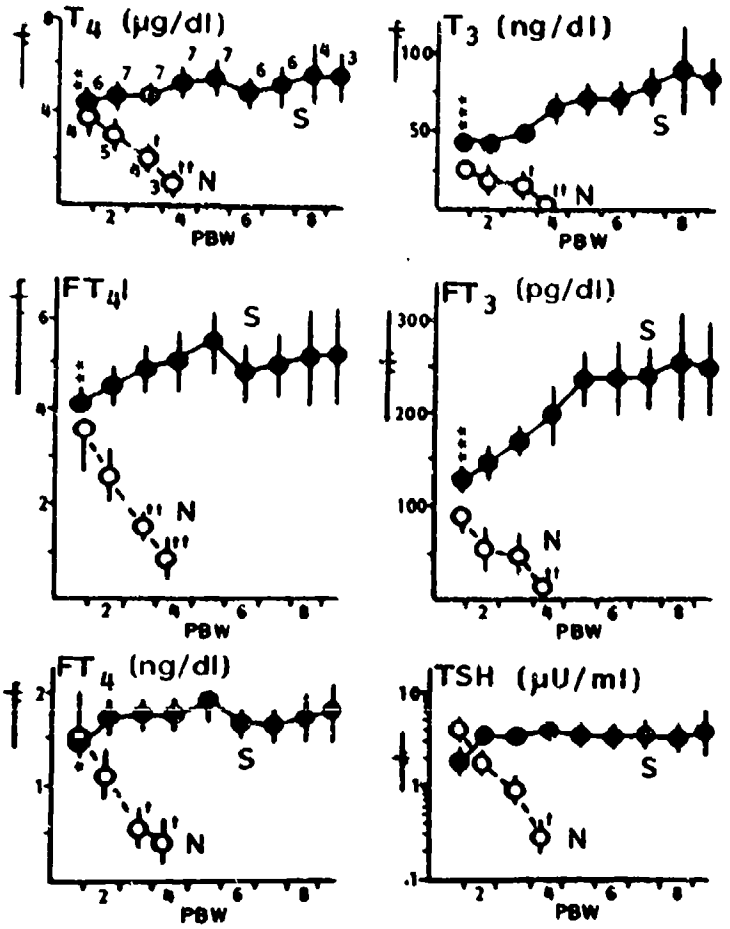


FIGURE 2. Mean ± standard error for thyroxine (T4), its free index (FT4I), its dialyzable concentration (FT4), triiodothyronine (T3), its dialyzable concentration (FT3), and thyrotropin (TSH) in surviving (S) and nonsurviving (N) burn patients. The configuration to the left of each ordinate represents the 10 normal controls: mean ± standard error (horizontal marks) and ±2 standard deviation (vertical extent). One control and one S are women, the others men. Data are the within-patient mean for a given postburn week (PBW), and the time-grouped numbers of patients are given in the T4 panel. Statistical comparisons among means were limited to respective S versus N, and S at PBW 1 versus controls. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus controls. †$P < 0.05$, ††$P < 0.01$ versus S.

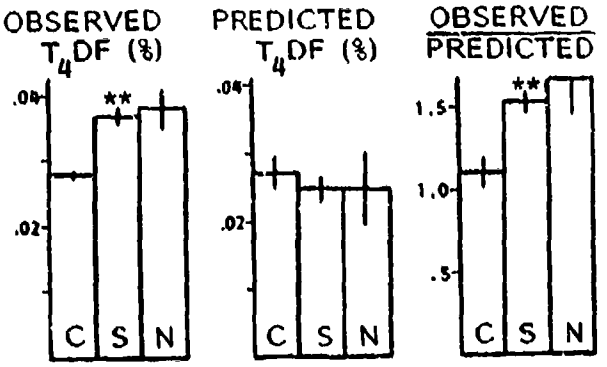


FIGURE 3. Observed thyroxine (T4) dialyzable fraction (T4DF), T4DF predicted on the basis of serum T4 and thyronine binding globulin, and the ratio (mean ± standard error) in controls (C), survivors (S), and nonsurvivors (N) of burn injury during the second postburn week. **P < 0.01 versus controls. For pooled survivors and nonsurvivors, the observed T4DF and the ratio were elevated (both P < 0.001) above the control mean.

In terms of molar concentration, x is bound T4 and TBG,

$$\left(\frac{x}{T4-x}\right)\left(\frac{1}{TBG}\right) = k\left(1 - \frac{x}{TBG}\right), \quad x = \frac{b - \sqrt{b^2 - 4a}}{2}$$

$$a = (T4)(TBG),\ b = 1/k + TBG + T4, \text{ and}$$

$$\text{percent predicted T4DF} = \frac{(T4 - x)}{T4}100$$

RESULTS

Preliminary results (Figure 1) indicated that in all of the 26 patients without burns and with elevated FT4I, serum TSH was below the normal range, a finding similar to that reported previously for this method (16).

Figure 2 indicates that mean serum total and free T4 and T3 in burn survivors were low in the first postburn week and subsequently rose toward the normal mean. Mean TSH in these patients was usually normal and exhibited occasionally minimally elevated values in some patients after the first postburn week. However, in nonsurvivors, these hormones progressively fell with time. In Table 2, the changes of rT3 were less impressive, with the mean in the third postburn week in nonsurvivors elevated above that for survivors. T4DF and

TABLE 2

ADDITIONAL SERUM VARIABLES

			CONTROLS	POSTBURN WEEK 1	2	3	4	5	6	7	8	9
rT3 (ng/dl)	Survivors	Mean	23.1	23.0	17.9	14.9	16.2	15.8	16.5	15.8	14.3	13.8
		Standard Error	1.3	2.0	1.6	0.9	1.1	1.3	1.4	1.4	1.4	1.1
	Nonsurvivors	Mean	-	30.4	27.4	23.6*	21.4	-	-	-	-	-
		Standard Error	-	2.1	4.9	1.7	4.5	-	-	-	-	-
T4DF (%)	Survivors	Mean	0.028	0.034**	0.037	0.037	0.036	0.035	0.035	0.033	0.032	0.032
		Standard Error	0.0004	0.002	0.001	0.001	0.001	0.001	0.002	0.002	0.002	0.001
	Nonsurvivors	Mean	-	0.039	0.038	0.048	0.052	-	-	-	-	-
		Standard Error	-	0.005	0.002	0.011	0.009	-	-	-	-	-
T3DF (%)	Survivors	Mean	0.222	0.305**	0.351	0.350	0.342	0.325	0.333	0.305	0.307	0.291
		Standard Error	0.010	0.015	0.018	0.013	0.008	0.015	0.017	0.017	0.026	0.005
	Nonsurvivors	Mean	-	0.353	0.344	0.344	0.505	-	-	-	-	-
		Standard Error	-	0.030	0.031	0.077	0.098	-	-	-	-	-
TBG (mg/dl)	Survivors	Mean	1.89	1.88	1.76	1.63	1.64	1.66	1.67	1.82	1.93	1.93
		Standard Error	0.11	0.13	0.13	0.04	0.05	0.08	0.09	0.15	0.24	0.07
	Nonsurvivors	Mean	-	1.39	1.85	1.54	1.32	-	-	-	-	-
		Standard Error	-	0.13	0.26	0.37	0.52	-	-	-	-	-
TG (ng/ml)	Survivors	Mean	4.57	4.03	4.51	4.43	5.14	5.14	6.02	6.90	4.98	-
		Standard Error	0.86	1.69	0.92	0.99	0.87	0.93	1.47	1.71	2.04	-
	Nonsurvivors	Mean	-	7.88	7.50	4.06	9.07	-	-	-	-	-
		Standard Error	-	3.45	1.98	1.13	4.71	-	-	-	-	-

NOTE: rT3 = reverse triiodothyronine, T4DF = thyroxine dialyzable fraction, T3DF = triiodothyronine dialyzable fraction, TBG = thyronine binding globulin, TG = thyroglobulin. The n for each group is given in Figure 2. *P < 0.05 versus controls, **P < 0.05 versus survivors, statistical comparisons of means being made only between respective survivors and nonsurvivors and between survivors at postburn week 1 and nonburned controls.

TABLE 3

CORRELATION COEFFICIENTS

	VERSUS TIME POSTBURN			VERSUS TSH		
	Survivors	Nonsurvivors	Slope Difference	Survivors	Nonsurvivors	Slope Difference
T4	0.56**	-0.54*	**	0.12	0.81***	**
FT4I	0.63**	-0.64*	***	0.21	0.78**	*
FT4	0.69***	-0.58*	***	0.28	0.84***	**
TSH	0.60**	-0.28	**	-	-	-

NOTE: Slope differences were tested by analysis of covariance. Samples were restricted to the first two weeks postburn. *P < 0.05, **P < 0.01, ***P < 0.001.

T3DF were elevated in burn patients and did not differ between survivors and nonsurvivors. Mean TBG and thyroglobulin were normal in survivors, not differing from those in nonsurvivors.

Table 3 shows that during the time when no dopamine was administered, the changes of serum TSH and T4 (total and free index and concentration) with time were positive in survivors and negative in nonsurvivors. T4 variables correlated slightly positively with TSH in survivors and significantly positively in nonsurvivors. The slopes with time or with TSH were significantly different between survivors and nonsurvivors.

Figure 3 indicates that while T4DF predicted on the basis of T4 and TBG values was not altered in burns, the observed T4DF and the observed/predicted ratio was elevated in burns. No difference between survivors and nonsurvivors was detected

DISCUSSION

Low concentrations of T4, particularly in nonsurvivors, confirm previous findings in burns (7,9-10) and other nonthyroidal illnesses (NTI) (21-22). Though previous reports in burn patients, with use of a TSH assay not validated for the normal to low range, suggested low (7) or normal (10) serum TSH in nonsurvivors, the pattern of circulating TSH after burn injury has not been clarified previously. We find parallel changes in TSH and T4-related measurements, but in opposite directions between survivors and nonsurvivors with time in the first two weeks after burn injury.

Interestingly, despite the above mentioned TSH changes and low thyronine concentrations, the mean TSH concentrations during both of the first two weeks were in the normal range in both survivors and nonsurvivors (Figure 2). Subsequent further fall of TSH after two weeks in nonsurvivors occurred in some cases even without dopamine infusion. The initially nondepressed TSH values suggest that something other than low TSH concentration contributes to the low levels of thyroid hormones seen early after burns. However, it is also possible that abnormal TSH secretion is present early, with a general inhibitory influence on the TSH-thyroid axis preventing more severe elevation of serum TSH. Our previous results (7) with use of a less sensitive and specific TSH assay showed that treatment with exogenous T3 lowered serum levels of T4, FT4I,

---

[21] Slag MF, Morley JE, Elson MK, et al: Hypothyroxinemia in critically ill patients as a predictor of high mortality. JAMA 245:43-45, 1981.

[22] Kaptein EM, Weiner JM, Robinson WJ, et al: Relationship of altered thyroid hormone indices to survival in nonthyroidal illnesses. Clin Endocrinol 16:565-574, 1982.

and TSH in survivors, but not the already lower levels in nonsurvivors. Without T3 treatment, nonsurvivors had a severely blunted response to TSH-releasing hormone. In survivors, the TSH response to TSH-releasing hormone was not augmented above normal, despite reduced thyronine levels (7). Thus, the thyrotrophes appear to remain responsive to low thyronine levels, but other factors may be suppressive for TSH secretion and interact to produce the different pattern between survivors and nonsurvivors.

The initial reduction in thyronine levels is not understood. Extravasation of plasma proteins (and presumably bound hormones) occurs in the burn wounds. However, the capillary leak is repaired in the first 24 to 48 hours after burn (23) and our observed results are not associated with reduced TBG levels. Thus, initial compartmental fluid shifts appear not to be a major factor in the reduced thyronine levels. In that clearance of thyronines from the distribution volume is accelerated in nonburned critically ill patients (24) and the free fractions of T4 and T3 are elevated in burn injury, increased clearance rate may have contributed to the depression of serum T4 and T3, and a reduced peripheral conversion of T4 to T3 generally associated with illness (24-28) may have contributed to the more severe depression of serum T3. Our samples were not taken frequently or early enough to identify possible decrements of immunoreactive TSH that might have occurred even before the initial changes in thyronines. Finally, whether postburn circulating TSH, when at normal immunoactive levels, might have a reduced bioactivity in burns is not yet known. This may be a reasonable hypothesis,

---

[23] Pruitt BA Jr, Mason AD Jr, and Moncrief JA: Hemodynamic changes in the early postburn patient: the influence of fluid administration and of a vasodilator (hydralazine). J Trauma 11:36-46, 1971.

[24] Kaptein EM, Robinson WJ, Grieb DA, et al: Peripheral serum thyroxine, triiodothyronine and reverse triiodothyronine kinetics in the low thyroxine state of acute nonthyroidal illnesses. J Clin Invest 69:526-535, 1982.

[25] Chopra IJ, Chopra U, Smith SR, et al: Reciprocal changes in serum concentrations of 3,3',5'-triiodothyronine (T3) in systemic illnesses. J Clin Endocrinol Metab 41:1043-1049, 1975.

[26] Cavalieri RR: Impaired peripheral conversion of thyroxine to triiodothyronine. Ann Rev Med 28:57-65, 1977.

[27] Wartofsky L and Burman KD: Alterations in thyroid function in patients with systemic illness: the "euthyroid sick syndrome". Endocr Rev 3:164-217, 1982.

[28] Chopra IJ, Hershman JM, Pardridge WM, et al: Thyroid function in nonthyroidal illnesses. Ann Intern Med 98:946-957, 1983.

because in burn patients with low basal T4, the reported serum T4 and T3 responses to injections of bovine TSH indicated responsiveness of the thyroid gland (8).

A general inhibitory influence on TSH secretion at the level of the pituitary might be expected in burn patients because of reported elevation in circulating levels of cortisol (29) and endogenous dopamine and other catecholamines (7,30). Since these elevations tended to be somewhat more exaggerated in nonsurvivors than in survivors, it is possible, though not demonstrated, that such a mechanism accounts for the difference between outcome groups now noted in the TSH-thyroid axis. In addition to inhibition at the level of the pituitary, there may be an inhibition of central nervous system stimulation of the pituitary, in that previous results showed a delayed TSH response to TSH-releasing hormone in nonsurvivors of burns (7). Further, effects of burn injury on the central nervous system in the rat model (burn size = 60 percent of the total body surface area) have been demonstrated. Whereas nonburned rats had marked elevations of serum TSH and whole brain T4-5'-monodeiodinase two weeks after thyroidectomy, nonthyroidectomized burned rats sampled two weeks after injury dramatically failed to exhibit any rise of TSH or deiodinase, though postburn serum T4 was suppressed to the same extent as seen in the thyroidectomized nonburned rats (14). Another rat study (13) showed that serum T4 is already depressed by six hours after burns and that after 48 hours, ectopic supraependymal neurons lining the ventricular wall of the hypothalamus emerged from the underlying tissue and remained for at least two weeks. A second plunge of serum T4 coincided with the appearance of the ectopically situated neurons. It was postulated that central nervous system changes contribute to suppression of the thyroid axis after burns.

In that mean serum TSH in the first two weeks postburn is not depressed below the normal range but changes correlate with falling serum T4 in nonsurvivors, it is likely that a positive influence on thyrotrophe function from low thyronine concentrations during this time is superimposed on negative influences from elevated endogenous serum dopamine and cortisol and/or from reduced hypothalamic stimulation. Such negative influences seem to become more prominant with time in nonsurvivors. In survivors, it is possible that less severity of these negative influences on TSH, resolving with time, but interacting with some inhibition of TSH from the rising serum

---

[29]Vaughan GM, Becker RA, Allen JP, et al: Cortisol and corticotrophin in burned patients. J Trauma 22:263-273, 1982.

[30]Wilmore DW, Long JM, Mason AD Jr, et al: Catecholamines: mediator of the hypermetabolic response to thermal injury. Ann Surg 180:653-669, 1974.

thyronines, prevented the positive correlation of T4 with TSH from reaching statistical significance in this group. In survivors of nonburn NTI, rises of serum TSH slightly above the normal range generally preceded the rise of T3 into the normal range (31). In burn and nonburn NTI survivors, very close interval sampling disclosed short-term rises in TSH slightly preceding rises in T4 (32). In patients with hematologic malignancies, low serum T4 was associated with reductions in TSH measured with a sensitive and specific assay (33). Our results indicate parallel changes in T4 and TSH, suggesting a role for TSH in the low T4 state associated with burn injury, and further show that TSH may be in the normal range early when the pattern with time already differs between survivors and nonsurvivors. These results appear to be independent of drugs known to influence the thyroid axis.

Though elevated serum rT3 has been reported in burn patients (2,6-7), this finding has not been consistent (5,8,10-11) or as dramatic as the changes in T3 (6-7). The skin of the rat normally contains much more T4 to rT3 converting activity and also a much higher rT3 concentration than any of several other tissues examined (34). In burned rats, serum rT3 is frankly depressed (14). If skin contributes to formation of rT3 also in humans, the elevation of serum rT3 characteristic of other forms of NTI (24-25,27-28) might be expected to be less prominent in burn patients with destruction of skin. Indeed, mean serum rT3 was not elevated in the burn patients of this study above the mean of controls, though in the third postburn week, the nonsurvivor mean was higher than the survivor mean. It is possible that organs other than skin contribute to rT3 production and/or reduced clearance in human NTI.

The observed depression in serum binding of T4 and T3 confirms previous results in burn injury (10-12). We now find that in burn patients this is not the result of a reduced concentration of immunoreactive TBG, the major binder of T4 and T3 in the human circulation. Smeds et al (6) have also reported normal TBG concentrations by radial immunodiffusion through most of the course after major burns, though they did

---

[31]Bacci V, Schussler GC, and Kaplan TB: The relationship between serum triiodothyronine and thyrotropin during systemic illness. J Clin Endocrinol Metab 54:1229-1235, 1982.

[32]Hamblin PS, Dyer SA, Mohr VA, et al: Relationship between thyrotropin and thyroxine changes during recovery from severe hypothyroxinemia of critical illness. J Clin Endocrinol Metab 62:717-722, 1986.

[33]Wehmann RE, Gregerman RI, Burns WH, et al: Suppression of thyrotropin in the low-thyroxine state of severe nonthyroidal illness. New Engl J Med 312:546-552, 1985.

not assess thyronine dialyzable fractions. Since the measured T4DF is partly a function of the concentrations of T4 and TBG, we calculated a predicted T4DF (on the basis of observed T4 and TBG and the normal association constant) and found it not to be elevated in burn patients. Since the measureable T4DF is additionally a function of the binding affinity and capacity of TBG and of other thyronine binding proteins and the concentrations of T4-binding prealbumin and of albumin, reduction of one or of a combination of these additional factors may explain the elevated observed/predicted T4DF ratio in burn patients. Reduced concentrations of T4-binding prealbumin (6,35) and albumin (9,36) have been reported after burns, and reported elevations of fatty acids (37), probably a result of the sustained elevations of circulating catecholamines, may contribute to reduced thyronine binding to TBG. Whether the binding characteristics or association constant of TBG are altered in burn injury has not been determined.

Elevation of the T4DF in NTI has been known for a long time (38). Because serum concentrations of thyronine binding proteins are sometimes normal or insufficiently reduced to explain the degree of reduced thyronine binding in nonburn NTI patients (38-40), there has been a search for a NTI-induced

---

[34]Huang T-S, Chopra IJ, Beredo A, et al: Skin is an active site for the inner ring monodeiodination of thyroxine to 3,3',5'-triiodothyronine. Endocrinology 117:2106-2113, 1985.

[35]Moody BJ: Changes in the serum concentrations of thyroxine-binding prealbumin and retinol-binding protein following burn injury. Clin Chim Acta 118:87-92, 1982.

[36]Davies JWL: Physiological Responses to Burning Injury. New York: Academic Press, 1982, pp 355-356.

[37]Davies JWL: Physiological Responses to Burning Injury. New York: Academic Press, 1982, pp 486-491.

[38]Oppenheimer JH, Squef R, Surks MI, et al: Binding of thyroxine by serum proteins evaluated by equilibrium dialysis and electrophoretic techniques. Alterations in nonthyroidal illness. J Clin Invest 42:1769-1782, 1963.

[39]Chopra IJ, Teco GNC, Nguyen AH, et al: In search of an inhibitor of thyroid hormone binding to serum proteins in nonthyroid illnesses. J Clin Endocrinol Metab 49:63-69, 1979.

[40]Woeber KA and Maddux BA: Thyroid hormone binding in nonthyroid illness. Metabolism 30:412-416, 1981.

binding inhibitor (39-45). It appears that the inhibitor is ether-extractable (41) and exhibits activity similar to that of nonesterified fatty acids such as oleic, linoleic, linolenic, and arachidonic, which inhibit binding of T4 to serum proteins, T4 antibody (41), TBG, and albumin (46) and inhibit hepatic T4-to-T3 conversion (47). Recent results (45) suggest that a small elevation of nonesterified fatty acids would significantly reduce serum binding of T4 in NTI and that susceptibility to the effect of nonesterified fatty acids is enhanced by reduced concentrations of albumin. A factor in NTI serum inhibits binding of T4 to incubated hepatocytes (42,44) and red cells (43). Kinetic studies in NTI patients suggested a hampered distribution of thyronines out of the circulation into tissues, despite reduced serum binding (24). Transhepatic extraction studies indicated elimination of net uptake of FT4 in the splanchnic bed of some burn patients (4). Such studies indicate that a thyronine binding defect in NTI and burns is a generalized phenomenon in more than one system. Thus, it is not surprising that altered serum thyronine binding after burns does not depend upon a change in concentration of TBG.

---

[41]Chopra IJ, Huang TS, Hurd RE, et al: A competitive ligand binding assay for measurement of thyroid hormone-binding inhibitor in serum and tissues. J Clin Endocrinol Metab 58:619-628, 1984.

[42]Oppenheimer JH, Schwartz HL, Mariash CN, et al: Evidence for a factor in the sera of patients with nonthyroidal disease which inhibits iodothyronine binding by solid matrices, serum proteins, and rat hepatocytes. J Clin Endocrinol Metab 54:757-766, 1982.

[43]Mendel CM and Cavalieri RR: Red blood cell thyroxine in nonthyroid illness and in heparin-treated patients. J Clin Endocrinol Metab 58:1117-1124, 1984.

[44]Sarne DH and Refetoff S: Measurement of thyroxine uptake from serum by cultured human hepatocytes as an index of thyroid status: reduced thyroxine uptake from serum of patients with nonthyroidal illness. J Clin Endocrinol Metab 61:1046-1052, 1985.

[45]Mendel CM, Frost PH, and Cavalieri RR: Effect of free fatty acids on the concentration of free thyroxine in human serum: the role of albumin. J Clin Endocrinol Metab 63:1394-1399, 1986.

[46]Tabachnick M and Korcek L: Effect of long-chain fatty acids on the binding of thyroxine and triiodothyronine to human thyroxine-binding globulin. Biochim Biophys Acta 881:292-296, 1986.

[47]Chopra IJ, Huang TS, Beredo A, et al: Evidence for an inhibitor of extrathyroidal conversion of thyroxine to 3,5,3'-triiodothyronine in sera of patients with nonthyroidal illnesses. J Clin Endocrinol Metab 60:666-672, 1985.

PRESENTATIONS/PUBLICATIONS

Scott DE, Vaughan GM, and Pruitt BA Jr: Hypothalamic neuroendocrine correlates of cutaneous burn injury in the rat: I. Scanning electron microscopy. Brain Res. Bull 17:367-378, 1986.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL (DD-DRAR) 636
				DA311508	86 10 01	
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISS'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BD	305
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY87-01		

11. TITLE (Precede with Security Classification Code) (U) Inequality of VA/Q Ratios Following Smoke Inhalation Injury and the Effect of Angiotensin Analogues

12. SUBJECT AREAS
06 05 Clinical Medicine 06 16 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 10	CONT	DA	C

MILITARY RELEVANCY CERTIFIED
APPROVED BY

17. CONTRACT/GRANT	18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE / EXPIRATION	a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER	86	0.0	0
c. TYPE / d. AMOUNT	87	2.0	65
e. KIND OF AWARD / f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: SHIMAZU, T
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-7832
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): IKEUCHI, H g. NAME OF ASSOCIATE INVESTIGATOR (If available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Inhalation Injury; (U) Cardiac Output; (U) Indicator Dilution; (U) Ventilation-Perfusion Ratio; (U) Cobra Venom;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (U) Lab Animals: (U) Sheep; (U) RAII

23. (U) To evaluate the effect of smoke inhalation on pulmonary ventilation and perfusion. To study the effects of positive end-expiratory pressure and oxygen on pulmonary ventilation-perfusion ratio. To study the role of the complement system in the respiratory insufficiency following smoke inhalation.

24. (U) The ventilation-perfusion ratio will be measured utilizing the six-inert gas technique. These pulmonary variables will be correlated with standard cardiopulmonary variables before and after the introduction of inhalation injury and subsequent treatment with positive end-expiratory pressure and/or oxygen. Lung lymphatic collection will be used to permit assessment of the pathophysiologic mechanisms of pulmonary edema formation and tho contribution of chemical mediators, especially platelet activating factors, to such edema formation after smoke inhalation. Animals decomplemented by cobra venom factors will be studied to assess the role of the complement system in the process of inflammatory responses following smoke inhalation.

25. (U) 8510 - 8609. Ventilation-perfusion alterations following smoke inhalation injury have been established. Techniques for lung lymph collections and blood platelet activating factor preparation have been standardized. This project was transferred from DA302498.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE. U.S. G.P.O. 1984-421-646/17001

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA311499	86 10 01	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BD	306
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY87-01		

11. TITLE *(Precede with Security Classification Code)* (U) Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model

12. SUBJECT AREAS
06 01 Biochemistry 06 13 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 10	CONT	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY

a. DATE EFFECTIVE		18. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
c. TYPE	d. AMOUNT	86	0.0	0
e. KIND OF AWARD	f. CUM/TOTAL	87	1.8	55

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)* Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR SHIPPEE, R L
d. TELEPHONE NUMBER *(Include area code)* 512-221-2720	d. TELEPHONE NUMBER *(Include area code)* 512-221-7138
21. GENERAL USE FINA MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR *(If available)* WILSON, S W g. NAME OF ASSOCIATE INVESTIGATOR *(If available)* KING, N L

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Thermal Injury; (U) Zinc Homeostasis; (U) Immunocompetence; (U) Lab Animals; (U) Rats; (U) RAII

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23. (U) The main objective of this research is to determine the changes in zinc metabolism caused by burn and infection in a murine model. The experiments are designed to study the effect of injury on physiological control mechanisms at the whole body, organ, and molecular levels. Information obtained form these studies will provide a better understanding of the postinjury changes in the metabolism of this important trace element and the role of these changes in septic complications, ultimately leading to the development of optimal supplementation of zinc in burned humans.

24. (U) To determine endogenous fecal and urine losses after burn injury, a semi-purified zinc deficient (> 0.5 ppm) diet will be fed to rats. The zinc supplemented groups will be given a daily subcutaneous zinc injection (one milligram/kilogram body weight). Total fecal and urine will be collected for 10 days after the rats are given a 30-percent full-thickness burn. After receiving the burn injury, the burn group will be divided up into zinc-supplemented and zinc-nonsupplemented groups. Three nonburned control groups will also be used, i.e., zinc sufficient/ad libitum fed, zinc sufficient/pair fed, and zinc deficient. Ten days postburn, the rats will be sacrificed and peripheral blood lymphocytes isolated and stained using fluorecein labeled monoclonal antibodies.

25. (U) 8510 - 8609. Experiments are being planned using the cell sorting capabilities of the flow cytometer to separate out lymphocyte

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE. — U.S. G.P.O. 1984-421-646/17001

CONTINUATION OF DD FORM 1498 FOR "PRELIMINARY STUDIES OF ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL"

subpopulations for use in various functional tests of immunocompetence. This project was transferred from DA305253.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA304527	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DRA&E(AR) 636
3. DATE PREV SUM'RY 85 10 01	4. KIND OF SUMMARY K	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISP'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	075
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE *(Precede with Security Classification Code)*
(U) Cardiovascular and Endocrine Sequelae of Burn Resuscitation

12. SUBJECT AREAS
06 16 Physiology 06 09 Hygiene and Sanitation

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 08	86 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY: Basil A Pruitt
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
86	0.2	3
87	0.0	0

19. RESPONSIBLE DOD ORGANIZATION

a. NAME
US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*
Fort Sam Houston
San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL
PRUITT, B A
d. TELEPHONE NUMBER *(Include area code)*
512-221-2720

20. PERFORMING ORGANIZATION

a. NAME
US Army Institute of Surgical Research
b. ADDRESS
Fort Sam Houston
San Antonio, Texas 78234-6200
c. NAME OF PRINCIPAL INVESTIGATOR
MASON, A D
d. TELEPHONE NUMBER *(Include area code)*
512-221-7832
f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*
HERSON, M R
g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*
VAUGHAN, G M

21. GENERAL USE
FINA
MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Resuscitation Fluids; (U) Burn Injury; (U) Hormones; (U) Cardiovascular Hemodynamics; (U) Lab Animals; (U) Rats;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

22. (Continued) (U) Sheep; (U) ILIR; (U) RAII

23. (U) This study is designed to assess the resuscitation needs in rats with 50-percent total body surface area burns. Central arterial pressure will serve as a guide to adjustments in infusion rates of fluids containing varying amounts of crystalloid and colloid solutions. Measurements of various hemodynamic indexes, blood chemistries, vasopressin, and renin angiotensin concentrations will be carried out at specific postburn intervals.

24. (U) This project assessed the effects of pure plasma loss upon the cardiovascular system and the resulting hypovolemic shock in animals subjected to continuous plasma loss effected through the use of a hemofiltation device.

25. (U) 8510 - 8609. Cardiodynamic responses to rapid plasma loss effected with a plasmapheresis filter were studied in unanesthetized adult sheep. The filters, interposed in previously inserted arteriovenous shunts, permitted continuous removal of a fluid almost identical to plasma without removal of formed blood elements. Filter flow rates, determined by systemic blood pressure, resulted in withdrawal of 94 to 105 percent of estimated plasma volume and an 88-percent increase in hematocrit prior to death. Higher rates of plasma removal were obtained initially, with subsequent decrease as hemoconcentration progressed. Maximum total plasma

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE. ☆U.S. G.P.O. 1984-421-646/11001

CONTINUATION OF DD FORM 1498 FOR "CARDIOVASCULAR AND ENDOCRINE SEQUELAE OF BURN RESUSCITATION"

loss and death occurred approximately two hours after initiation of plasma removal. Animal behavior, systemic blood pressure, heart rate, pulmonary artery pressure, pulmonary wedge pressure, cardiac output, and peripheral resistance were monitored, and blood biochemical and gas concentrations were measured. All animals developed severe metabolic acidosis without a respiratory component. The hemodynamic responses were similar to those in hemorrhagic shock except for significant increases in hematocrit and blood osmolality. Survival time appeared to be determined by rate of plasma loss, but also varied with individual tolerance to the procedure. With slight modification, this model will permit simulation of burn shock of varying severity and should be of value in exploring both compositional and temporal responses to resuscitation. The results of such studies also pertain to other instances of shock in which decrement of plasma volume occurs, ranging from crush injury to acute peritonitis, and thus may have wide utility for all forms of trauma.

RESEARCH COMPLETION REPORT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: CARDIOVASCULAR AND ENDOCRINE SEQUELAE OF BURN RESUSCITATION: Hemodynamic Effects of Controlled Pure Plasma Loss in Sheep

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Marisa R. Herson, MD
Arthur D. Mason, Jr., MD

ABSTRACT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: CARDIOVASCULAR AND ENDOCRINE SEQUELAE OF BURN RESUSCITATION: Hemodynamic Effects of Controlled Pure Plasma Loss in Sheep

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

INVESTIGATORS: Marisa R. Herson, MD
Arthur D. Mason, Jr., MD

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

Cardiodynamic responses to rapid plasma loss effected with a plasmapheresis filter have been studied in five unanesthetized adult sheep. The filters, interposed in previously inserted arteriovenous shunts, permitted continuous removal of a fluid almost identical to plasma without removal of formed blood elements. Filter flow rates were determined by systemic blood pressure and resulted in withdrawal of 94 to 105 percent of estimated plasma volume and an 88-percent increase in hematocrit prior to death. Higher rates of plasma removal were obtained initially, with subsequent decrease as hemoconcentration progressed. Maximum total plasma loss and death occurred approximately two hours after initiation of plasma removal. Animal behavior, systemic blood pressure, heart rate, pulmonary artery pressure, pulmonary wedge pressure, cardiac output, and peripheral resistance were monitored, and blood biochemical and gas concentrations were measured. All animals developed severe metabolic acidosis without a respiratory component. The hemodynamic responses were similar to those described in hemorrhagic shock except for significant increases in hematocrit and blood osmolality. Survival time appeared to be determined by rate of plasma loss, but also varied with individual tolerance to the procedure. With slight modification, this model will permit simulation of burn shock of varying severity and should be of value in exploring both compositional and temporal responses to resuscitation.

HEMODYNAMIC EFFECTS OF CONTROLLED PURE PLASMA LOSS IN SHEEP

INTRODUCTION

The objectives of this study were to assess the effects upon the cardiovascular system of rapid, continuous plasma loss. Severe volume deficits in animals and the associated hemodynamic responses have usually been studied by depleting animals of whole blood to known, reproducible end points, with or without controlled fluid replacement. These models reflect the physiological behavior of most hemorrhagic shock syndromes. The loss of whole blood, however, represents an intravascular volume depletion with a simultaneous decrease in red cell mass. Red cells are not only oxygen carriers and important buffer system elements, but also play a very important role in blood viscosity and coagulation. The individual effects of impairment of oxygen delivery, changes in blood rheology, and of volume deficit per se can only be indirectly evaluated in such models.

The postburn hypovolemic state is a result of both fluid loss to the environment through the burned tissue and leakage of fluid into the extravascular-extracellular space (so-called "third space"). Studies have shown that this fluid, in both circumstances, is isotonic with plasma and has similar concentrations of albumin and sodium (1).

The increase in efflux of plasma through the capillary membranes and edema formation after burn injury has been reported to be the result of increased capillary hydrostatic pressure, of increased oncotic extracellular pressures in the injured tissues due to collagen denaturation, of increased capillary permeability, and of decreased intravascular oncotic pressure (2-4). The ultrastructural basis for normal capillary permeability has been the object of many studies. In

---

[1]Baxter CR: Fluid volume and electrolyte changes of the early postburn period. Clin Plas Surg 1:693-709, 1974.

[2]Arturson G: Microvascular permeability to macromolecules in thermal injury. Acta Physiol Scand (Suppl) 463:111-122, 1979.

[3]Arturson G: Pathophysiological aspects of the burn syndrome with special reference to liver injury and alterations of capillary permeability. Acta Chir Scand (Suppl) 274:1-135, 1961.

[4]Arturson G and Mellander S: Acute changes in capillary filtration and diffusion in experimental burn injury. Acta Physiol Scand 62:457-463, 1964.

1951 Pappenheimer (5) introduced the concept of pores (90 A in diameter) and slits (40 A in diameter) in the capillary membranes. Later on, Grotte (6) and Mayerson (7) postulated the additional presence of a small number of larger openings (250-500 A in diameter). These pores were described as more numerous in the venous capillaries and venules while rare in the arterial capillaries and arterioles (8). Other mechanisms involved to a lesser extent in the passage of substances across capillary walls are vesicular transport and, in normal circumstances, transfer through intercellular junctions that may function like small pores.

In traumatized animals (e.g., burns), changes in tissue antigenicity and osmolality and liberation of mediators trigger processes which result in immediate diffuse changes in the capillary membrane. When inflammation takes place, widening of the intercellular junctions occurs, creating gaps in the endothelium. This phenomenon is most prominent at the postcapillary level (2,9). Some relation may exist between this finding and the fact that pores are more numerous in the venular side to begin with (10). When such gaps are formed, the basement membrane initially acts like a filter, retaining larger molecules, but then microscopic ruptures (11) and small tunnels created by the migration of leukocytes render the

---

[5]Pappenheimer JR, Renkin EM, and Borrero LM: Filtration, diffusion and molecular sieving through peripheral capillary membranes. A contribution to the pore theory of capillary permeability. Am J Physiol 167:13-46, 1951.

[6]Grotte G: Passage of dextran molecules accross the blood-lymph barrier. Acta Chir Scand (Suppl) 211:1-84, 1956.

[7]Mayerson HS, Wolfram CG, Shirley HH Jr, et al: Regional differences in capillary permeability. Am J Physiol 198:155-160, 1960.

[8]Landis EM: Heteroporosity of the capillary wall as indicated by cinematographic analysis of the passage of dyes. Ann NY Acad Sci 116:765-773, 1964.

[9]Cotran RS: The fine structure of the microvasculature in relation to normal and altered permeability. In Reeve, E. B., and Guyton, A. C. (eds).: Physical Bases of Circulatory Transport: Regulation and Exchange, Philadelphia, WB Saunders Company, 1967, pp 249-275.

[10]Majno G, Palade GE, and Schoefl GI: Studies on inflammation. II. The site of action of histamine and serotonin along the vascular tree: a topographic study. J Biophys Biochem Cytol 11:607-626, 1961.

[11]Cotran RS, La Gattuta M, and Majno G: Studies on inflammation. Fate of intramural vascular deposits induced by histamine. Am J Pathol 47:1045-1077, 1965.

passage of macromolecules possible (12). The end result is a transudation of plasma into the extravascular compartment and edema formation. Hypovolemia ensues, eventually progressing to shock and correspondent hemodynamic changes.

An Extracorporeal Filtration Device as a Tool for Continuous Plasma Removal. Hemofiltration filters are composed of hollow membranes with variable pore sizes. Such devices allow the sieving of different components from blood flowing through them into a filtrate compartment (Figure 1).

Plasmapheresis (i.e., plasma removal with return of corpuscles) has been used in clinical practice since 1914 (13) as an important adjunct in the treatment of diseases where removal of unwanted substances from the blood can improve the patients' condition. The principles and technical details of the procedure have been published (14-16). At first, the blood was withdrawn, centrifuged, the plasma separated, and the red cells returned. More recently, continuous centrifugal processing has been used. At present, filters composed of perforated hollow membranes encased in a plastic filtrate reservoir are available. Depending on the selected diameter of the membrane "pores," water, electrolytes, and protein molecules may be sieved in different percentages.

The PLASMAFLO AP-05H ASAHI[R] plasma separator (Parker Hannifin Corporation) used in this experiment is composed of approximately 3,000 hollow cellulose diacetate fibers with pores of 0.2 microns in diameter. The effective filtration surface is 0.5 m^2. This filter is highly permeable to free water, electrolytes, and blood macromolecules (including

---

[12]Hurley JV: Acute inflammation: the effect of concurrent leucocyte emigration and increased permeability on particle retention by the vascular wall. Brit J Exp Path 45:627-633, 1964.

[13]Abel JJ, Rowntree LG, and Turner BB: Plasma removal with return of corpuscles (plasmapheresis). J Pharmacol Exp Ther 5:625-641, 1914.

[14]Lauer A, Saccaggi A, Ronco C, et al: Continuous arteriovenous hemofiltration in the critically ill patient. Ann Intern Med 99:455-460, 1983.

[15]Malchesky PS, Werynski A, Asanuma Y, et al: Clinical operation of Asahi plasma separators. Artif Organs 5(Suppl):113-116, 1981.

[16]Taft EG: Therapeutic apheresis. Human Pathol 14:235-240, 1983.

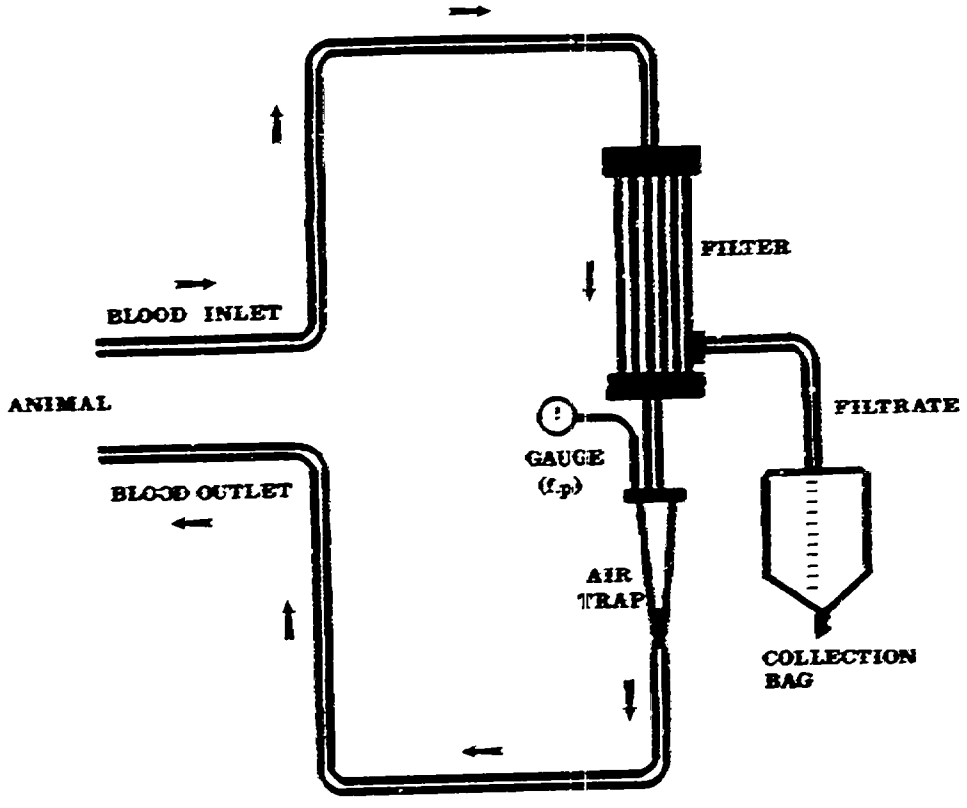


FIGURE 1. Graphic representation of the filtration circuit.

albumin), with total sparing of formed elements of blood (17) and produces a filtrate almost isotonic and isosmotic with plasma (Figure 2).

The filtration fraction (plasma filtered per volume of plasma that runs through the filter) is dependent on blood flow rate and on blood viscosity or hematocrit (Figure 3). The flow of blood enters the hollow fibers at an inlet pressure higher than that in the filtrate compartment (filtrate pressure); the pressure difference drives water and other molecules through the pores. Excessive transmembrane pressures (inlet pressure - filtrate pressure) can lead to plugging of pores by the red cells or to hemolysis. The higher the hematocrit, the less plasma per unit of volume running through the filter is accessible for separation. High viscosity also may increase the formation of cell aggregates and pore plugging.

A constant flow of blood through the filter can be effected by either the animal's systemic blood pressure as proposed by Kramer (18) or by the use of a pump with preset flow rates. In the present model, the animal's blood pressure was used as the driving force for the filtration process. We assume that the hollow fibers and their pores mimic the capillaries when permeability is increased.

MATERIALS AND METHODS

Neutered, random source, one to two-year-old male sheep weighing 27 to 34 kilograms (average = 30 kilograms) were used as experimental animals. Both sheep and dogs have been used as subjects for shock models in the past, permitting many hemodynamic and biochemical comparisons. An important factor necessitating the use of a larger animal was that the filters employed require relatively large blood volumes and flow rates to assure appropriate plasma filtration.

First Stage. After induction of general anesthesia with methohexital sodium (nine milligrams/kilogram body weight, Brevital® sodium, Lilly Laboratories), the animals were intubated. Adequate anesthetic levels were maintained with methoxyflurane (PENTHRANE®, Abbott Laboratories, Inc.). The

---

[17]Parker Hannifin Corporation: Clinical data from the Oji National Hospital, Tokio; Cleveland Clinic Foundation; Nagoya University Branch Hospital, Nagoya; Rush Presbyterian St. Lukes Medical Center, Chicago; and the University of Texas Health Sciences Center, San Antonio.

[18]Kramer P, Wigger W, Rieger J, et al: Arteriovenous hemofiltration: a new and simple method of treatment of overhydrated patients resistent to diuretics. Klin Wochenschr 55:1121, 1977.

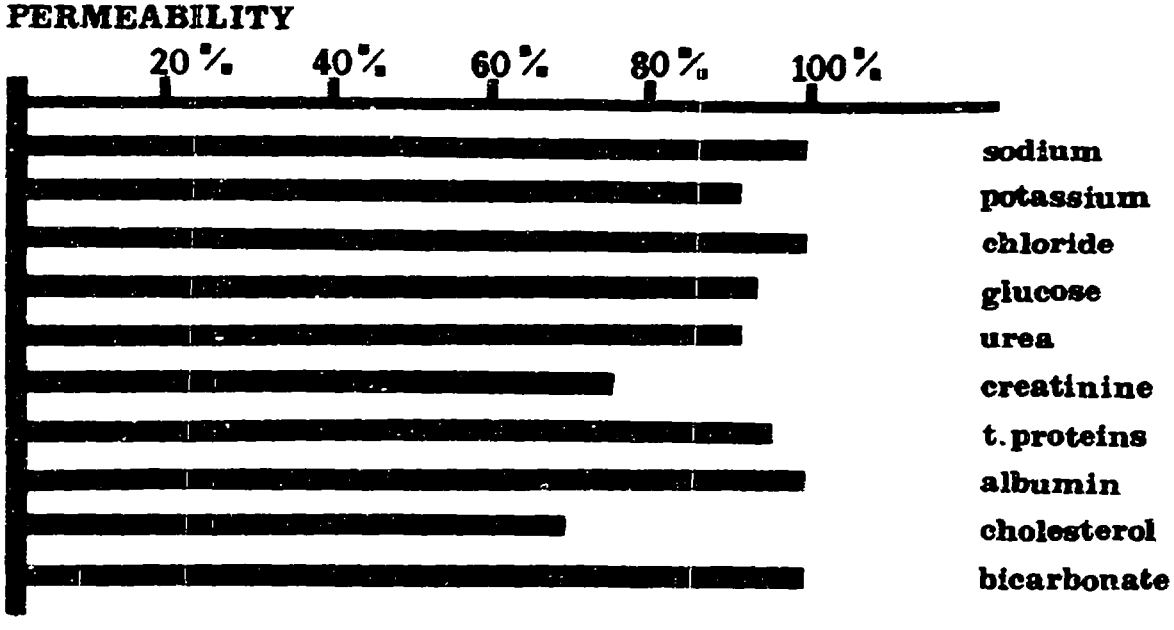


FIGURE 2. Sieving coefficients of the filter for various blood components.

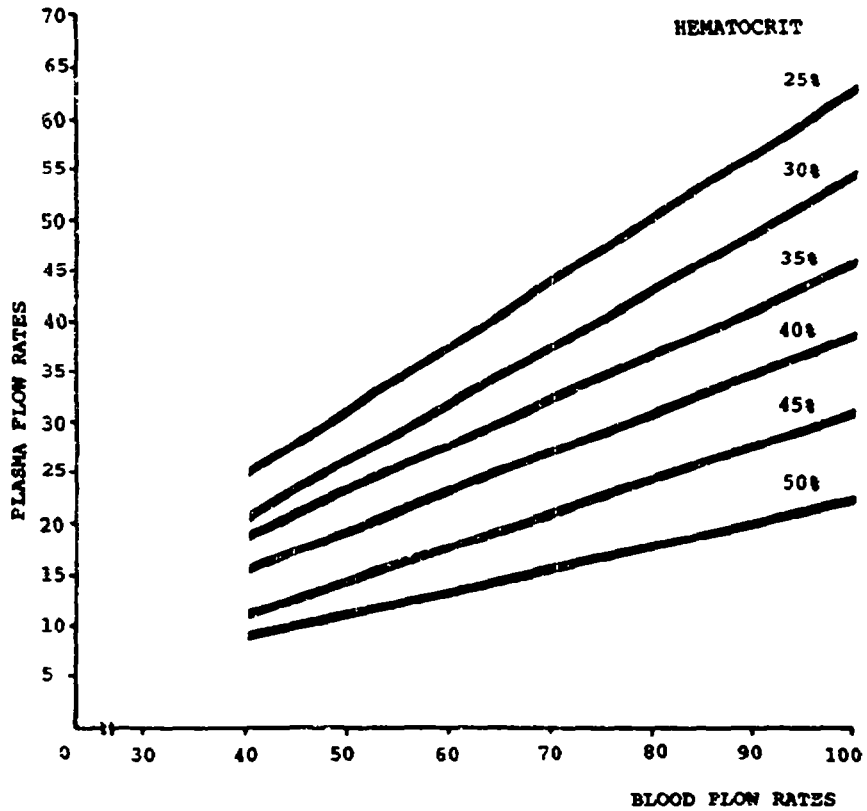


FIGURE 3. Relationship between hematocrit, blood flow, and plasma withdrawn shows that higher flows and lower hematocrits result in more efficient performance.

right femoral vein and artery were cannulated with silicone cannulae; a flow-directed thermodilution pulmonary artery catheter (7F Swan-Ganz catheter, American Edwards Company) was introduced through the left jugular vein.

Vascular access for ultrafiltration was established between the right external jugular vein and the right carotid artery. These vessels were chosen for cannulation to permit ease of manipulation and higher blood flows. Vascular access was accomplished with a modification of an arteriovenous shunt developed by Dennis, Cole, and Scribner in 1974 (19); a SAF-T-SHUNT® arteriovenous cannula (ID = 2.59 millimeters, Extracorporeal Medical Specialties, Inc.) was shortened and etched vessel tips (Extracorporeal Medical Specialties, Inc.) were placed at both ends. After introduction into the dissected vessels, the closed loop obtained was externally exposed through the animals' skin. This loop assured adequate arterial and venous access when severed in the second stage of the experiment.

The animals were then placed in restraint cages in isolated rooms and fed ad libitum for three to five days. The vascular lines were flushed every six hours with heparinized saline solution. All arteriovenous shunts were patent at the time of the second intervention. The animals received alternate daily doses of an aqueous suspension of penicillin (400,000 units) and hydrostreptomycin sulphate (0.5 grams).

Second Stage. Three to five days after cannulation, provided the animals were in clinically stable condition, the second part of the experiment took place. The animals were alert and sufficiently accustomed to their surroundings to allow for manipulation without noticeable changes in behavior or hemodynamic indices.

The animals were systemically heparinized (bolus dose, 1,000 units/kilogram body weight); anticoagulation was confirmed by measurement of the activated partial thromboplastin time. The heparinized status was maintained by constant infusion of heparin (25 units/kilogram body weight/hour). Central venous pressures and pulmonary artery pressures were monitored using Statham P23Db transducers and the systemic arterial pressure was monitored with a Hewlett-Packard 1290A Quartz transducer. These data were graphically recorded using a Hewlett-Packard 7754A four-channel recorder.

---

[19]Dennis MB Jr, Cole JJ, Scribner BH: Long-term vascular access for animal studies. J Appl Physiol 37:978-981, 1974.

A PLASMAFLO AP-05H^{R} plasma separator with shortened GAMBRO^{R} hemodialysis blood lines was primed with heparinized Ringer's lactate solution (5,000 units heparin/1,000 cubic centimeters solution). This sytem, with the plasma outlet clamped, was connected to the animal's circulation after sectioning the shunt loop. Initiation of blood flow through the system produced certain hemodynamic effects prior to plasma removal; these effects will be discussed. After these baseline hemodynamic measurements were completed, the plasma outlet was opened and plasma removal was started.

The standard filtration lines were shortened considerably. With high blood flows and a normal hematocrit, the length of these lines should not be a factor in hemodynamic changes. It was noted, however, that as the animals' blood pressure decreased and as the hematocrit values rose (higher blood viscosity), shorter lines allowed for prolonged adequate blood flow through the system.

Cardiac output measurements were performed at intervals with a 9520A Cardiac Output Computer (American Edwards Laboratories) along with arterial and mixed central venous blood gas determinations. Blood samples, hematocrits, and filtrate aliquots were also collected at intervals.

The studies were continued until the spontaneous death of the animals. Postmortem examinations were performed for animal quality control, morphologic evaluation, and tissue sampling.

RESULTS

Responses to filtrate removal without replacement were measured in five sheep. The average duration of the plasma loss experiments was two hours (one hour, 40 minutes to two hours, 40 minutes). These animals demonstrated very similar behavior and the data obtained are grouped for discussion. Two other experiments were terminated before the death of the animals because of intramembrane clotting of the filters. Although their behavioral patterns and hemodynamic changes were similar, the data obtained from these animals are omitted from this report.

Animal Behavior. As described before, all studies were conducted with the animals awake and with no sedation. There was no major suffering or pain experienced by the animals, although the animals were mildly agitated in the late stages of hypovolemia. The fact that the animals were not anesthetized allowed for observation of physical responses that changed the hemodynamic and filtration patterns; such observations would have been lost if an anesthetic agent had been employed.

All animals became less alert almost immediately after initiation of plasma withdrawal with attendant severe hypotension. Following this, the behavior of the animals followed a cyclic pattern. After 30 to 40 minutes, the animals "woke up," moving foward in the sling, and hyperextending their necks in a manner suggesting a Valsalva maneuver. Coincidently, blood pressures and filtration rates rose. By 50 minutes, the animals again relaxed, but remained responsive to stimuli. Another period of agitation then followed with slight increases in blood pressure and filtration rate. Then, although the animals became quiet again, shallow rapid breathing was noted for 10 to 15 minutes followed by a phase of slow, deep breathing. Concomitantly, higher filtration rates were again observed in most animals. The level of conciousness of the animals then progressively deteriorated and, approximately one and one-half hours after the beginning of filtration, the animals became unresponsive and without corneal reflexes. Bouts of generalized convulsions occurred in the three to five minutes prior to death.

Plasma Filtration Rates and Changes in the Hematocrit. The normal plasma volume for adult sheep is estimated to be in the range of 36 to 40 milliliters/kilogram (20). As can be seen in Table 1, the average total filtrate removed was 1,100 cubic centimeters, representing 94 to 105 percent of the initially calculated intravascular plasma volume. This amount of plasma was removed during an average of two hours. The filtration rates were not constant. As soon as the filtration process was initiated (Figure 4), rapid filtrate removal occurred (> 20 cubic centimeters/minute). During the 15 to 20 minutes that these high rates continued, almost 50 percent of the total filtrate volume was removed. The volumes removed thereafter were progressively smaller until filtration finally stopped, usually 15 to 20 minutes before the animals' deaths. It is interesting to note that in the later stages of the experiments, there was recurrent coincidence between agitation of the animals, increase in the systemic blood pressure, and small increases in the filtration rates. The curves obtained therefore show an initial filtration Peak 1 followed by two other distinct peaks. Peak 2 usually could be related to an increase in the animals' systemic blood pressure and Peak 3 coincided with the previously described phase of deep breathing. About 80 to 100 minutes after starting the filtration (10 to 25 minutes before death) and shortly after filtration ceased, spontaneous resorption of the filtrate remaining in the filter's chamber was noted.

---

[20]Fox JG, Cohen BJ, and Loew FM (eds). Laboratory Animal Medicine, Orlando, Academic Press, 1984, pp 282-285.

TABLE 1. Plasma Removal as to Estimated Initial Plasmatic Volumes

Animal	#1	#2	#3	#4	#5
Weight (kg)	30	29	27	30	30
Time lag (minutes)	160	115	100	115	125
Estimated plasma volume (cc) (36-40 cc/kg)	1080-1200	1044-1160	972-1080	1080-1200	1080-1200
Plasma Volume Removed (cc)	911	1557	919	1155	957
Plasma Volume Removed (%)	84.3-75.9	149.1-134.2	94.5-85.0	106.9-96.2	88.6-96.2

OVERALL MEAN PLASMA REMOVED (%) = 94.2-104.6

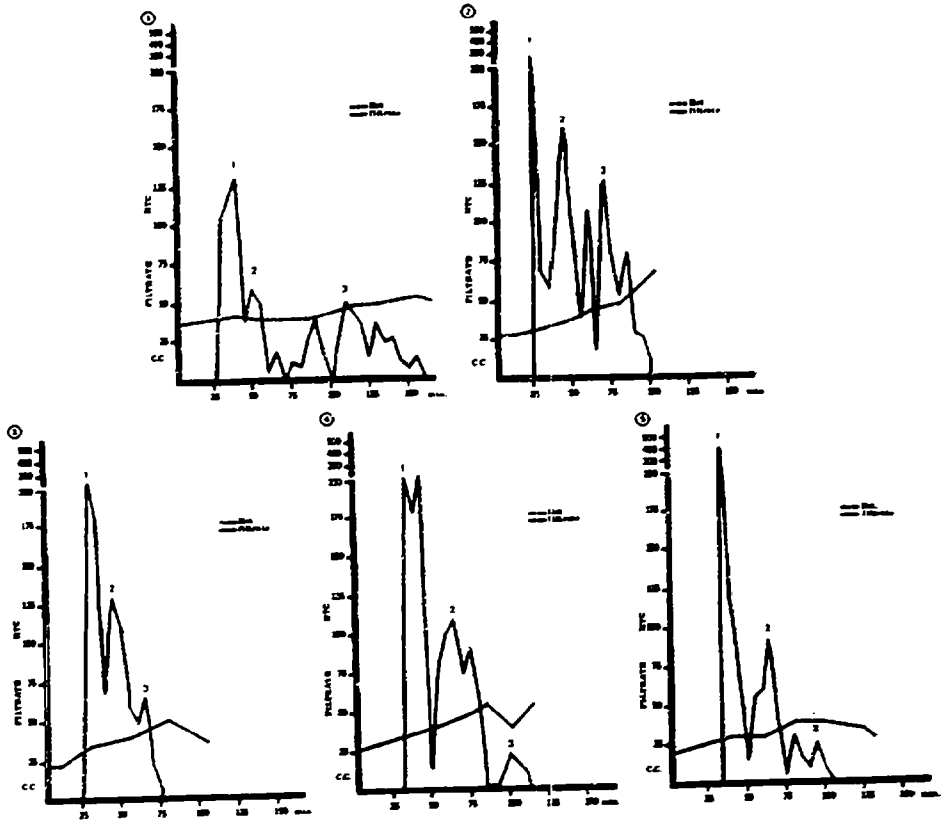


FIGURE 4. Curves show relationship of time and filtration rates. Point 1 refers to high volumes obtained soon after starting withdrawal. Points 2 and 3 refer to further rises in filtration rates coincident with changes in animals' behavior (deep breathing, motor agitation).

The normal range of the hematocrit in adult sheep is 24 to 40 percent (21). This wide range of normal values can be explained by the important role of the spleen as a red cell storage organ in these animals. Adrenergic stimulation causes the spleen to contract, with spillage of red cells into the circulation and increasing hematocrit (22). In our studies, the combination of both stimulation and plasma volume depletion led to a steady rise of the hematocrit with only a slight decrease in some of the animals at the very end of the experiment (Figure 4). During the experiments, there was an average 88.2-percent increase of the baseline hematocrit (Table 2). There was no clear relation between the volume of plasma removed, the initial hematocrit, and the percentage increase of the hematocrit. The decrease of the hematocrit in the final stages of the study coincided with resorption from the filtrate compartment and death of the animal.

Systemic Arterial Blood Pressure Changes and Variations of the Cardiac Rhythm. As the changes in both systemic arterial blood pressure and heart rate followed patterns that appear interrelated, the data obtained are analyzed together. Immediately after the filtration circuit was connected to the animals' circulation (Figure 5, point A), there was a small decrease in mean blood pressure, mainly as a consequence of a decrease in diastolic pressure (Figure 5, point 0). This decrease was followed by an increase of blood pressure almost to control levels. Within five minutes after initiating removal of plasma (Figure 5, point B), the mean blood pressure dropped sharply to 25 to 30 mmHg (Figure 5, point 1). This decrease was not only of mean blood pressure, but included a "pinching" of pulse pressure. The heart rate also decreased rapidly, and occasional sporadic bouts of arrythmia were observed in some animals. Still later, the pulse pressures widened and the mean blood pressure stabilized at hypotensive levels. Some very discrete increases in mean blood pressure (Figure 6, points 2 through 5) coincided with increases in plasma filtration rate (Figure 4, points 2 and 3). Heart rates then progressively increased to extraordinary levels (290 beats/minute) and finally decreased sharply in the last few minutes of the animals' lives.

As described, a clear sequence of responses was observed after plasma withdrawal was started, abrupt hypotension and bradycardia, hypotension and tachycardia, hypotension with

---

[21] Hecker JF: The Sheep as an Experimental Animal, London, Academic Press, 1983.

[22] Demling RH, Harms B, Kramer G, et al: Acute versus sustained hypoproteinemia and posttraumatic pulmonary edema. Surgery 92:79-86, 1982.

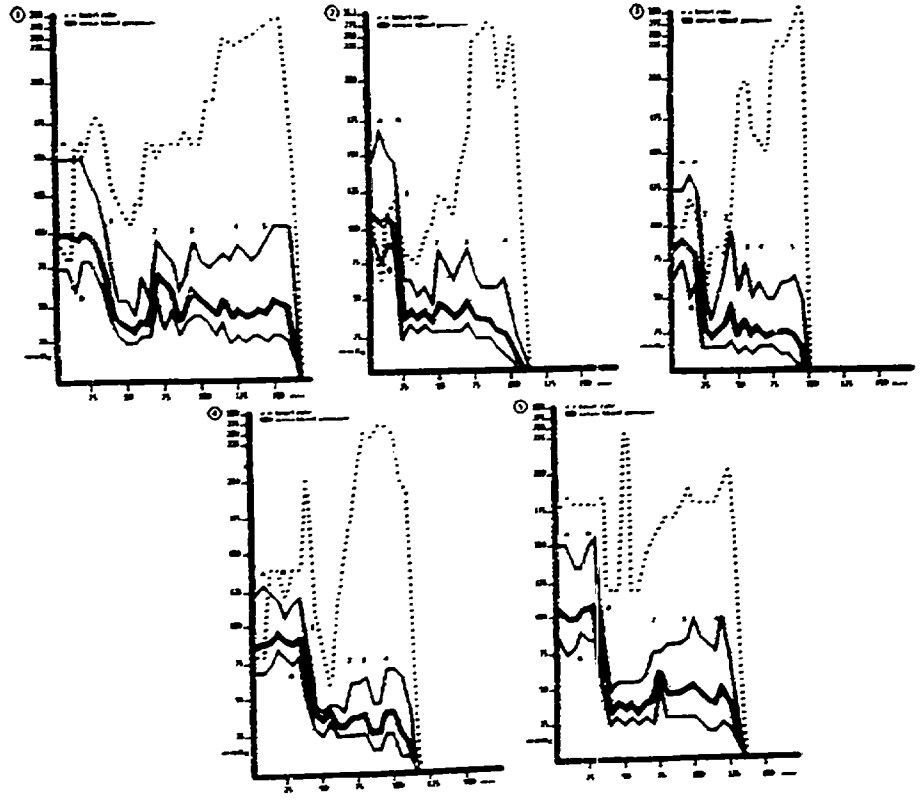


FIGURE 5. Continuous line represents mean blood pressure and dotted line heart rate. Point A indicates beginning of blood flow through circuit; point B indicates beginning of plasma removal. 0 refers to the initial drop in blood pressure when fistula was opened and 1 to the severe drop soon after beginning of plasma withdrawal. 2, 3, 4, and 5 are further increases in blood pressures coincident with changes in animals' behavior.

TABLE 2. Hematocrit Value Changes (Percentage)

Animal	#1	#2	#3	#4	#5
Initial Hematocrit	35.5	28.5	24.5	26.0	22.0
Final Hematocrit	52.0	72.0	49.0	48.0	35.0
Increase	46.4	152.0	100.0	84.0	59.0

OVERALL MEAN HEMATOCRIT INCREASE = 88.2

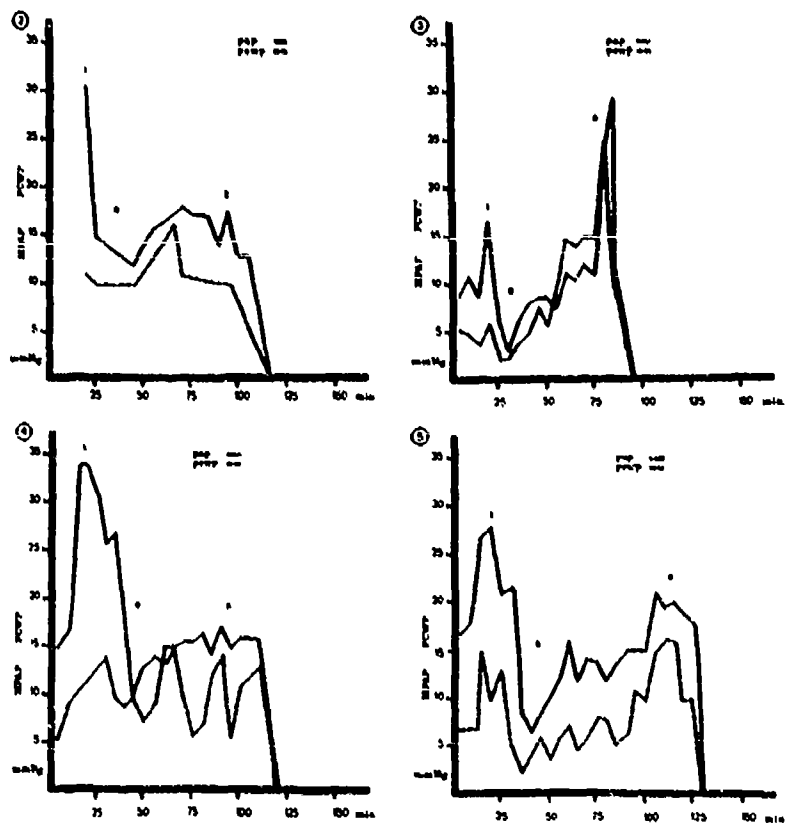


FIGURE 6. Mean pulmonary artery pressures (PAP) and pulmonary capillary wedge pressures (PCWP). Point 1 represents the significant increase in the mean PAP immediately following the opening of the circuit. As soon as filtration was started, the values decrease (point 2). Point 3 represents a final rise in values preceding the animals' death.

almost normal heart rates, and finally hypotension and severe tachycardia followed by death of the animals.

Mean Pulmonary Artery Pressure Curves and Changes in Pulmonary Wedge Pressures. Although not as clearly as in the systemic blood pressures, a trend could also be observed in the mean pulmonary artery pressure (MPAP) curves (Figure 7). Soon after the connection of the filtration circuit and opening of the shunt, there was a significant increase in pulmonary arterial pressure (Figure 6, point 1). A return to approximately baseline MPAP occurred within 20 minutes. When plasma withdrawal was initiated, a decrease in MPAP coincided with the severe initial hypotension (Figure 6, point 2). After a subsequent moderate increase, the pressures then remained almost constant for most of the the experiment. Another late rise preceded the animals' death (Figure 6, point 3).

The mean pulmonary capillary wedge pressure (PCWP) curves had a similar configuration (Figure 6). In two animals in the later stages of the experiments, the values of MPAP and PCWP were almost equal. Also, at random times, the tracings of the MPAP and PCWP would look very similar (Figure 7). This effect could be seen during short intervals of time (10 to 15 minutes), after which the normal differences between the tracings could be again observed. No clear relation could be found between the appearance of these patterns and changes of MPAP, respiratory rate, mean systemic blood pressure, or pulse pressure, time lag or volume of plasma removed, or the death of the animal.

Cardiac Output Curves and Changes in the Peripheral Vascular Resistance. As can be seen in Figure 8, with the exeption of animal #4, cardiac output did not change significantly after the arteriovenous fistula was opened and the blood started flowing through the filtration circuit. After plasma withdrawal was started, however, cardiac output fell steadily until the animals' death.

The peripheral vascular resistance (PVR) was calculated using the formula:

$$PVR(dynes.sec.cm\text{-}5) = \frac{(AP[mmHg] - CVP[mmHg]) \times 79.9}{\text{Cardiac Output (liters/minute)}}$$

and the results are shown in Figure 9. In animal #4, the PVR showed a transient and modest decrease shortly after plasma removal was initiated. Otherwise, the values increased gradually until the end of the studies.

Biochemical Studies of Filtrate and Blood. The studies of the filtrate showed consistent filtration coefficients

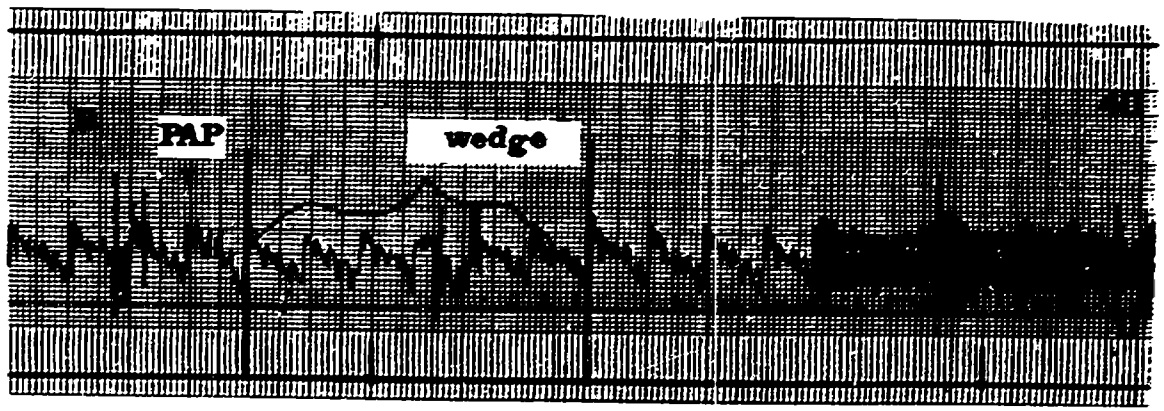


FIGURE 7. The pulmonary arterial and wedge pressures are recorded in this strip. Note the extremely similar pattern of both the pulmonary arterial and wedge pressures. The animal had a systemic blood presure of 70/20 mmHg.

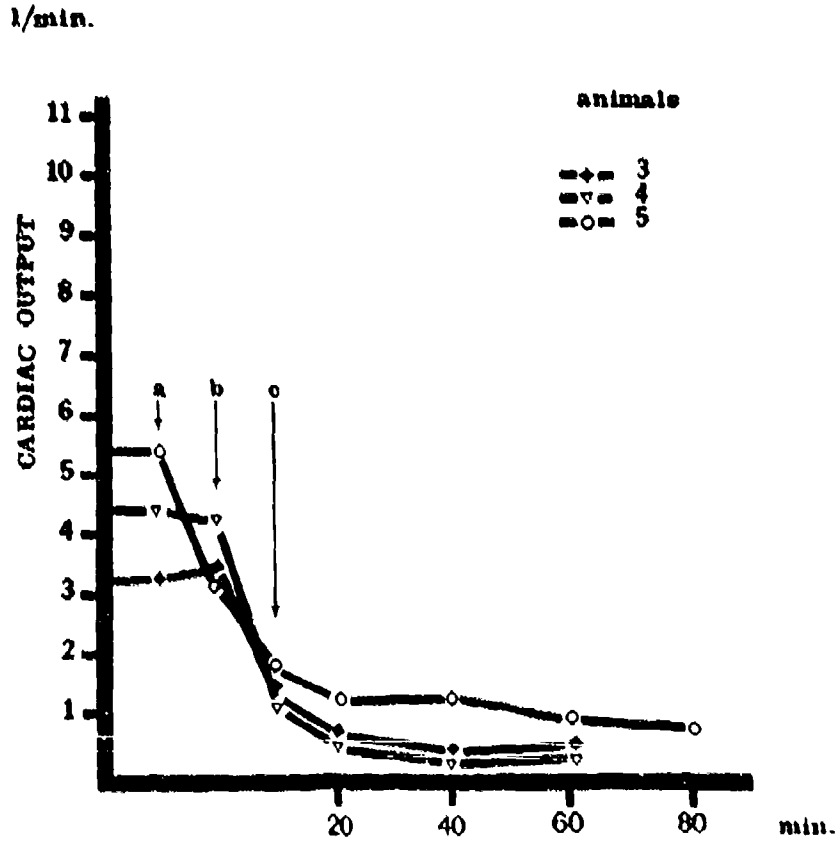


FIGURE 8. Tendency for decrease in values of cardiac output can be appreciated. a = control values, b = after blood flows through circuit, and c = after initiation of plasma withdrawal.

independent of the animals' hematocrit and blood viscosity. The approximate sieving coefficients obtained were Na 100%, K 92%, Cl 100%, glucose 94%, BUN 80%, creatinine 76%, albumin 100%, and bicarbonate 100%.

The changes in serum sodium, potassium, blood urea nitrogen, creatinine, glucose, bicarbonate, and osmolality values can be appreciated in Figure 10. Note that the glucose concentration increased significantly. In contrast, bicarbonate and albumin concentrations decreased considerably from control values.

Arterial and Central Mixed Venous Blood Gases. Progressive acidosis with severe lowering of both arterial and venous blood pH was a striking common event (Figure 11). There were concomitant decreases in the base excess values and bicarbonate

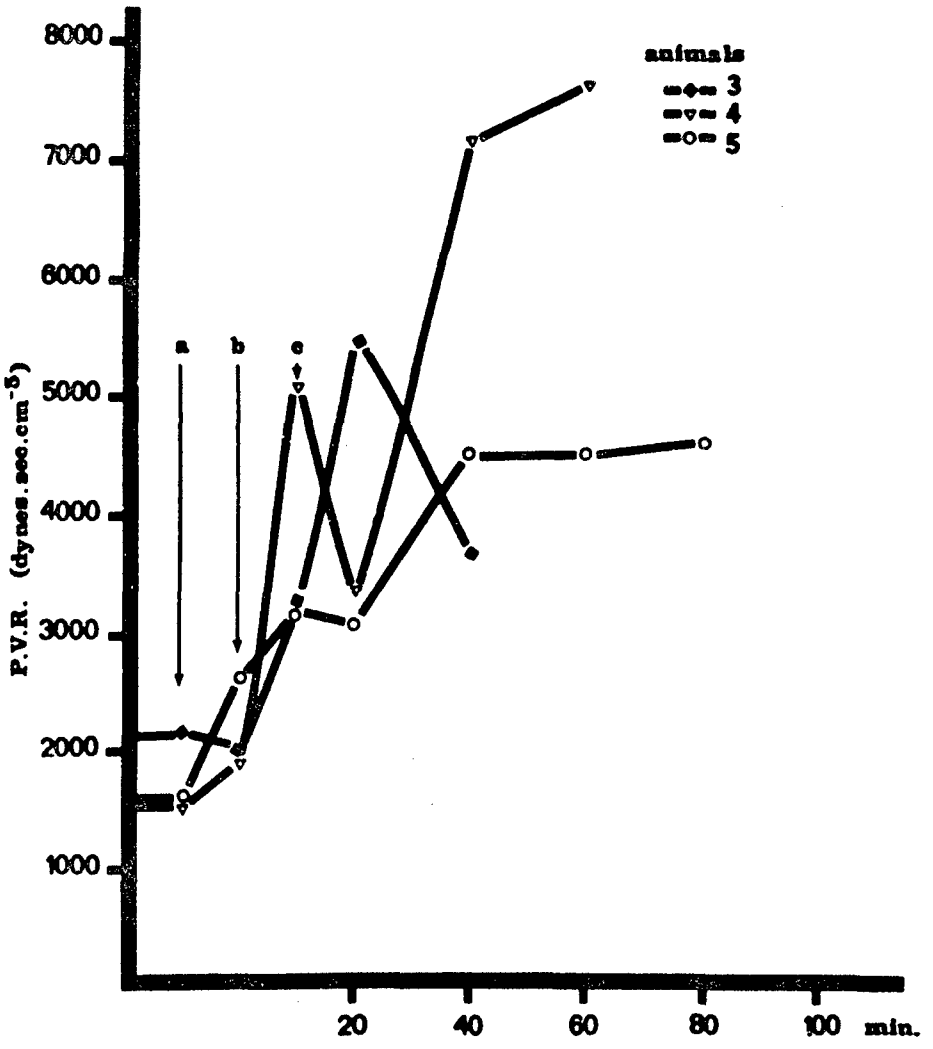


FIGURE 9. In an almost opposite way, the peripheral vascular resistance increases. a = control values, b = after blood flows through circuit, and c = after initiation of plasma withdrawal.

concentrations. Mixed venous blood concentrations of bicarbonate were higher than those found in the arterial samples (mean = 1.84 milliequivalents/liter). Increases in arterial oxygenation and decreases in carbon dioxide partial pressure levels coincided with the animals' tachypnea late in the experiment. In summary, these data demonstrate a progressive, predominantly metabolic acidosis.

Postmortem Morphological Examinations. Four animals were examined. Macroscopically, the contents of the abdominal cavity were intact, and in only one animal, a small amount (approximately 100 cubic centimeters) of clear intra-abdominal fluid was found. The livers were not significantly enlarged although the intrahepatic vessels were dilated. On sectioning,

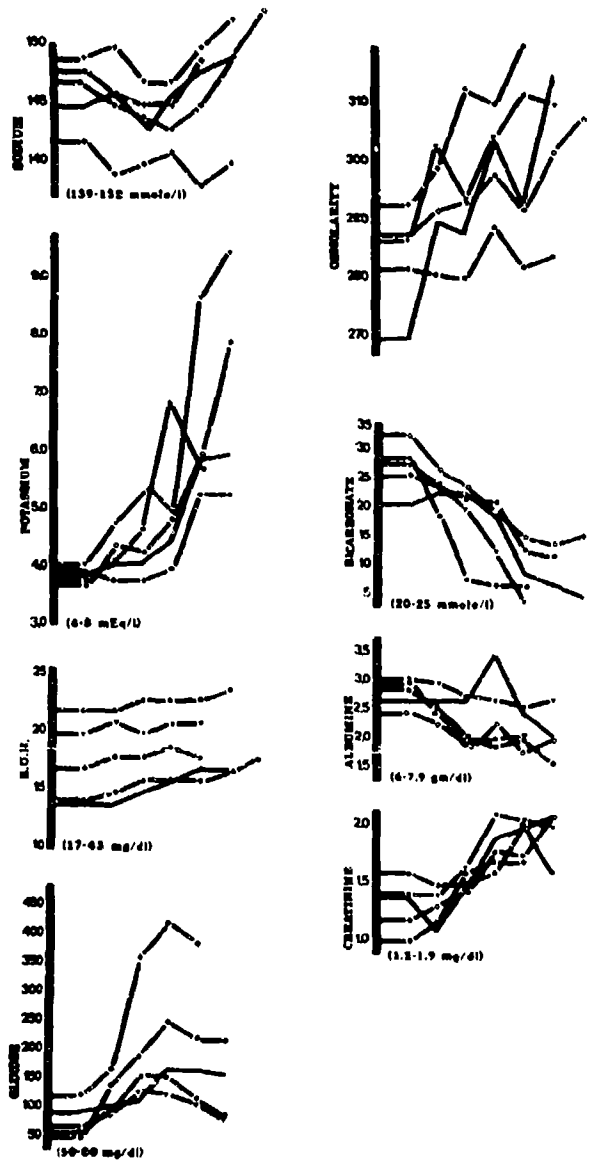


FIGURE 10. Electrolyte and serum protein changes during experiment. Normal values for sheep are depicted in bottom part of each graph.

the kidneys were engorged but without necrotic areas. The adrenal glands looked normal. The gut was normal in macroscopic appearance throughout its length, without signs of mucosal or intraluminal hemmorhage. The thoracic cavity revealed collapsed and congested lungs with signs of

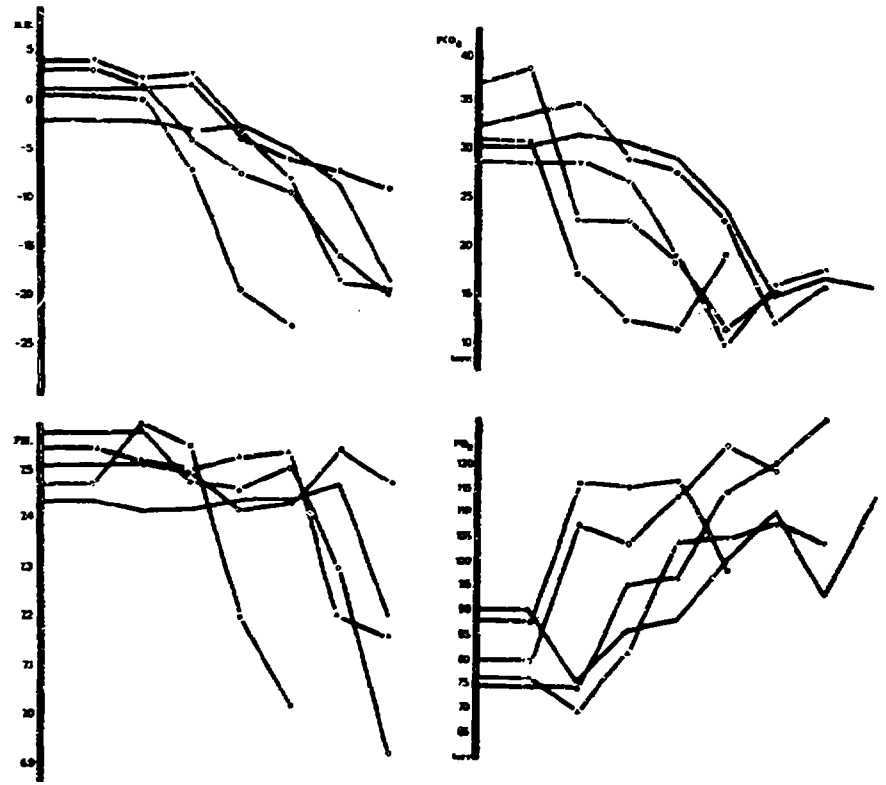


FIGURE 11. Progressive metabolic acidosis can be appreciated by comparison of graphs. Note significant drop in pH values in late stages of experiments independent of the fact that decreases of base excess begin early in the course. Decrease in pH coincides with rise in carbon dioxide levels, reflecting a preterminal failure of ventilatory compensation for metabolic acidosis. There is no clear explanation for the different initial patterns of the oxygen curves.

hepatization and focal areas of hemorrage. The intrapulmonary vasculature was very dilated and congested. The hearts were not enlarged but upon sectioning of the chambers, focal areas of endocardial necrosis and hemorrhage could be observed (agonal focal necrosis). Intracranial examinations were not performed.

The most constant microscopic finding was diffuse congestion of all organs, especially of the lungs. Some small areas of hemorrhage and atelectasis were also present. In one specimen, moderate hepatic centrilobular anoxic changes could be seen. Very few inflammatory cells were identified in any of the microscopic slides.

DISCUSSION

The plasmapharesis hemofilter proved a reliable tool for continuous removal of fluid with an electrolyte and protein content almost identical to plasma and appears practical for the study of the cardiovascular responses consequent to such loss. Previous similar studies of removal of plasma from experimental animals have relied upon a process of blood withdrawal, centrifugation, separation of the formed elements, and their return to the animals' circulation with a significant time lag between the removal of blood and return of the red cells to the circulation. In our experiments, hemodynamic changes occurred very quickly with little time for extravascular fluid shifts to modify the response patterns.

The clearest clinical example of intravascular fluid depletion with little significant loss of formed elements of blood in humans is an extensive burn. Although the decrease of the red cell mass in patients with burns over 64.5 percent of the total body surface area was 8.85 percent of initial mass/day (23), different variables interfere in the calculation of the average plasma volume loss/unit of burn in the first 24 hours after injury (e.g., anatomical distribution, depth of injury, etc.). The calculation of the rate of plasma loss/unit time or plasma loss/unit of burned tissue in humans and animals has been the subject of various studies. In 1951 Brooks (24) reported a loss of one milliter/percent burned area in dogs during the first six hours postburn. The Evans formula, based

---

[23]Pruitt BA Jr, Mason AD, and Moncrief JA: Hemodynamic changes in the early postburn patient: the influence of fluid administration and a vasodilator (hydralazine). J Trauma 11:36-46, 1971.

[24]Brooks JW, Robinett P, Largen TL, et al: A standard contact burn; method of production and observations on the blood picture following its production in dogs. Surg Gyn Obst 93:543-554, 1951.

on Brooks' studies, replaces two milliliters/kilogram body weight/percent burned area (25). Michie (26) observed a 20-percent decrease in the circulating intravascular volume within six hours after a standard 80-percent body surface area scald burn in dogs. Kilgore et al (27) described a reduction of 25 percent in plasma volume in burned (35 to 40 percent) rhesus monkeys within 18 hours as well as a decrease of 10.5 percent in the red cell mass and a loss of 44 percent of the functional extracellular fluid. Other values mentioned in the literature have been indirectly derived from the clinical response to different volumes of fluid replacement during resuscitation. In 1947 Cope and Moore (28) assessed a 25 milliliter/percent burn area/24 hours requirement for resuscitative volume in adult burned patients. The Parkland formula is based upon an estimated requirement for four milliliters/kilogram body weight/percent burned area. Pruitt et al (23) studied 10 patients (mean total body surface area burned = 64.5 percent) who required average fluid replacement of 10 milliliters/kilogram body weight/hour for the first four to six hours postburn and 4.4 milliliters/kilogram body weight/hour thereafter.

To estimate the relation between the extent of the capillary leak that occurs following burn injury and the present experimental volume loss, we may consider the data from Pruitt's work as universal for any burn independent of its distribution, extent, and depth. If we assume an average body weight of 70 kilograms and a baseline intravascular plasma volume of 3.5 liters, the calculated fluid lost from the intravascular space to the extravascular compartment within 24 hours in these resuscitated patients should approach 2.5 times the estimated preburn intravascular plasma volumes. Then a 64.5 percent burn in a 30-kilogram sheep might result in a loss of approximately 3,840 cubic centimeters in 24 hours (250 percent of the estimated intravascular volume) or 440 cubic centimeters in two and one-half hours. We obtained rates of plasma sieving at least three times higher. If the filter's effective filtration surface (0.5 m^2) is considered as a burned

---

[25]Evans EI, Purnell OJ, Robinett PW, et al: Fluid and electrolyte requirements in severe burns. Ann Surg 135:804-817, 1952.

[26]Michie DD: Cardiodynamic and hemodynamic events which occur following thermal injury, Galveston, Texas Graduate School, 1966, Dissertation.

[27]Kilgore E, Baxter CB, and Shires GT: Changes in body fluid compartments in full thickness burns. Surg Forum 16:29-31, 1965.

[28]Cope O and Moore FD: The redistribution of body water and the fluid therapy of the burned patient. Ann Surg 126:1010-1045, 1947.

area for a 30-kilogram animal and using a two cubic centimeter/kilogram body weight/percent burn area resuscitation formula, a loss of 3,000 cubic centimeters/24 hours, or 312.5 cubic centimeters in two and one-half hours, would be expected. Again, the rates obtained were approximately three times the calculated values. Since the rapidity of plasma volume loss in this model probably influenced the hemodynamic changes that occurred, the observed patterns of change can be viewed as an accelerated model of burn injury.

The rate of plasma loss into the extravascular space is not constant after trauma. Studying the results of scalding a cat's paw, Arturson (2,29) determined a 300-percent increase in microvascular permeability within five to 10 minutes after injury with a high rate of edema formation (20 to 40 milliliters/minute/liter/minute/mmHg/100 grams of tissue). He also observed the maximal increase in capillary permeability between one and three hours postburn, with a later decrease in net transudation rate as the blood flow within the paw lessened. Moore (30) considered the rate of loss of plasma to be accelerated during the first eight to 12 hours postburn, assuming an exponentially decreasing curve. In our model, the curves of plasma removal also assumed a configuration similar to that described by the mentioned authors, i.e., an initial peak followed by an exponential decrease.

The initial high plasma filtration rate was related to normal mean blood pressures. The later decrease in filtration rate coincided with lower mean blood pressures and higher blood viscosity. Further transient increases in filtration occurred simultaneously with small peaks in mean systemic pressure and higher blood flow rates through the system that occurred either spontaneously or when the animal responded with deep breathing. Deep breathing decreases intrathoracic and increases intra-abdominal pressures; both changes facilitate higher venous return and improvement in cardiac output. When the hematocrit reached critical levels (100-percent increase from baseline values), these maneuvers lost their efficiency and no plasma filtrate was obtained. Both high viscosity and the tendency of platelets and fibrin to obstruct the pores of the membranes in low flow states could cause diminution of capillary filtration *in vivo*.

The use of this artificial device in the animals required full heparinization to avoid intramembrane clotting and pore

---

[29]Arturson G: Pathophysiology of acute plasma loss in burns. *Bibl Haemat* 23:1130-1135, 1965.

[30]Moore FD: The body-weight burn budget. Basic fluid therapy for the early burn. *Surg Clin North Am* 50:1249-1265, 1970.

obstruction. Some authors (31-32) have postulated that a combination of high viscosity and low flow would result in the sedimentation of formed elements of blood and intravascular coagulation (blood sludging). Crowell (33) mentions a role of excessive amounts of lactic acid in neutralizing heparin locally. Microvascular dilatation may initially overcome this impediment, allowing for flow around clusters of cells, but once this response becomes ineffective because of futher aggregation, the delivery of essential metabolites to tissue cells is impaired, culminating in cell failure and death. Our use of heparin might have altered these effects. One alternative is the use of regional heparinization, i.e., heparin administered directly into the arterial blood inlet port, with neutralization by protamine sulphate at the filter outlet port. This technique requires the use of a blood pump in the circuit to obtain precise blood flow rates so that adequate dosages of heparin and protamine may be determined. Since such limitation of flow was not desired in the model at this stage, the viable alternative was our use of systemic heparinization.

The introduction of an arteriovenous shunt into an animal's circulation leads to immediate and delayed specific effects on cardiac output and systemic vascular resistance. The venous return to the heart is regulated in part by systemic vascular resistance. When this resistance decreases, venous return to the heart increases, leading to higher stroke volumes and increased cardiac output (34). Seconds after the opening of a shunt, stimulation of baroreceptors leads to the liberation of mediators and compensatory vasoconstriction. A compensatory increase in mean systemic blood pressure occurs and heart rate increases. When a large arteriovenous fistula is opened, higher heart rates have a significant role in increasing

---

[31] Knisely MH, Bloch EH, Elliot TS, et al: Sludged blood. Science 106:431, 1947.

[32] Wells RE Jr: Rheology of blood in low flow states. In Mills LC and Moyer JH (eds): Shock and Hypotension: Pathogenesis and Treatment: the Twelfth Hahnemann Symposium, New York, Grune & Stratton, c1965.

[33] Crowell JW: The influence of shock on the clotting mechanism. In Conference on Recent Progress and Present Problems in the Field of Shock, Washington, DC, Walter Reed Army Institute of Research, 1960.

[34] Guyton AC, Jones CE, and Coleman TG: Peripheral vascular contribution to cardiac output regulation - the concept of "venous return." In Circulatory Physiology: Cardiac Output and Its Regulation, Philadelphia, WB Saunders Company, 1973, pp 173-187.

cardiac output values (35-36). Within a variable time, delayed regulatory mechanisms supervene, leading to both increased intravascular volume and cardiac hypertrophy.

In our experiments, the animals went through a three to five-day adaptation period after the arteriovenous fistulas were created. It can be assumed that any acute compensatory mechanisms should have diminished and be of little significance at the time of the actual experiments. As a matter of fact, there was a uniform one to two-percent decrease in hematocrit between the earliest hematocrit measurements and the control hematocrits on the experimental day. Whether this was a result of a compensatory intravascular volume increase after the placement of the fistula or simply better animal hydration is not known. Within a few minutes after starting flow through the system without filtration, a very discrete increase in heart rate was noted (Figure 12). More significant were the changes in the arterial blood pressures; a slight decrease was followed in a few seconds by an increase, the values then returning to baseline. The most striking feature, however, was an almost twofold increase of pulmonary artery pressure and pulmonary capillary wedge pressure. Cardiac output increased briefly in only half of the animals. It may be that the compensatory mechanisms in sheep are quite efficient and the effects of changes in venous return are lost within seconds. The animals did not show any signs of physical distress at this stage and after five to 25 minutes, all parameters did return to approximately baseline values. These changes may simulate those resulting from the numerous microshunts that theoretically occur at tissue level after an extensive burn.

As soon as plasma removal was begun, responses to hypovolemia occurred, i.e., decrease in the systemic blood pressure and compensatory tachycardia. Vasoconstriction (as a response to hypovolemia) and the increasing blood viscosity (higher hematocrit) resulted in progressively higher peripheral resistance values. Venous return was impaired and a vicious circle of low venous return and even lower cardiac outputs ensued. It has been noted by Pareira (37) that when rats are bled to severe hypotensive levels, blood volumes return to control levels within four hours. Theoretically, with enough time, an animal could compensate for such intravascular volume depletion and shock by absorption of extracellular fluid. The

---

[35] Burton AC: Physiology and Byophisics of Circulation, Chicago, Year Book Publishers, c1965.

[36] Cowley AW and Guyton AC: Heart rate as a determinant of cardiac output in dogs with arteriovenous fistula. Am J Cardiol 28:321-325, 1971.

[37] Pareira MD, Serkes KD, and Lang S: Plasma volume response to graded hemorrhage. Surgery 52:378-381, 1962.

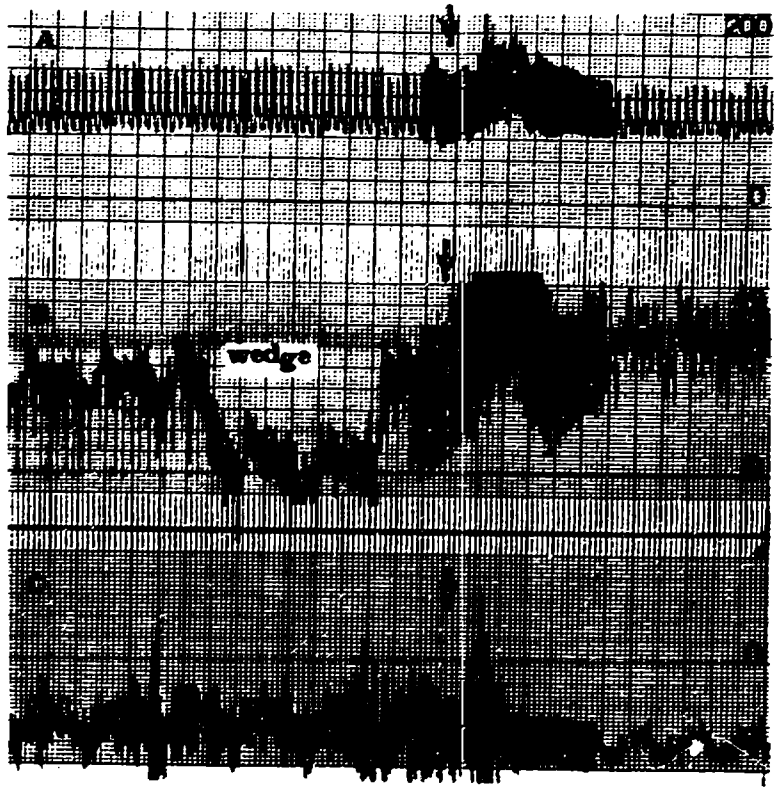


FIGURE 12. Hemodynamic effects at beginning of flow of blood through the filtration circuit can be clearly seen, moderate rise in blood pressure (arrow, strip A), significant rise in mean pulmonary arterial pressure values (arrow, strip B), and slight rise in central venous pressure (arrow, strip C). All values tended to return to control values within minutes.

beginning of the irreversible phase of shock occurs when this capacity for intrinsic replenishment is exceeded by the rate of volume loss. Very low flow rates with plugging of arterioles and venules would then occur and the consequent interference with the microscopic circulation would cause hypoxia, the accumulation of undesirable metabolites at cellular level (e.g., lactic acid), and finally, due to severe impairment of the cellular metabolism, cell death.

Two elements appeared to determine survival time in these animals, the rate of plasma removal and the animal's intrinsic tolerance for the procedure. One animal not included in the data reported here presented normal control hematocrit and albumin values and underwent plasma volume depletion, but survived for a comparatively long time (seven and one-half hours). Although the initial hemodynamic behavior was similar to that in the reported animals, the filtration continued at low rates during a much prolonged interval and there was no significant increase in the hematocrit, suggesting a more balanced fluid volume restoration. By replenishing the intravascular space, this animal was able to maintain better peripheral perfusion for a longer period of time. Only when these compensatory mechanisms failed, as reflected in a lowering of the arterial blood pH, did the animal die.

Changes in the blood pH seem to reflect the metabolic situation and the animals' compensatory capability best. All our animals died when their arterial blood pH reached extremely acidotic levels (pH = 7.0). In animal #3, very low pH values were reached surprisingly early in the study and death, too, was early. Increasing carbon dioxide partial pressure levels and decreasing base excess reflected a progressive anaerobic pattern of peripheral metabolism. The phase of shallow rapid breathing probably represents compensatory hyperventilation. As an aside, the higher values of bicarbonate observed in the mixed venous samples appear to reflect the difference in buffering capacity between oxygenated and reduced hemoglobin (38).

Most changes in the electrolyte content of the plasma reflect progressive volume depletion and hemoconcentration. Rises in creatinine and blood urea nitrogen reflect compromise of the renal blood flow. As in other forms of trauma, the combination of inhibited insulin secretion, impairment of its peripheral effects, and the secretion of cortisol, cathecolamines, and glucagon lead to increasing levels of blood

---

[30]Davenport HW: The ABC of Acid-Base Chemistry, Chicago, University of Chicago Press, c1958.

glucose (39). This increase in glucose may contribute to the higher blood osmolality, which could be an important force for compensatory intravascular volume increase, an osmotic force attracting intracellular water into the extracellular and intravascular space. The mechanisms for the observed increases in blood osmolality are not entirely clear; some of the increases are incompatible with the concentration changes in the measured plasma components.

Many works in the literature concerning hemorrhagic shock (40-42) describe a stage of "reversal of circulation," corresponding to the spontaneous resorption of shed blood from the reservoir back into the animal's circulation. It usually occurs within 60 to 80 minutes and, according to some authors (40), establishes the beginning of the irreversible phase of shock. One explanation for this phenomenon is failure of vasoconstriction with consequent increase in vascular capacitance, resulting in lower intravascular pressure and resorption. In our model such a change may be obscured by presence of the adynamic filter in the circulation, making resorption evident only terminally and when the plasma deficit is so great that filtration ceases. In these experiments, usually only 10 to 15 minutes preceeding the animals death and simultaneously with the cessation of filtration, diminished pressures in the filtrate reservoir were noted. If plasma were available, plugging of membrane pores by clusters of red cells may further diminish filtration at this time and the rise in osmolality may contribute to the influx of fluid into the vessels from the filtrate compartment.

This model has shown that volume loss without concomitant reduction of the red cell mass generates hemodynamic changes very similar to those observed in hemorrhagic shock. Blood pressure, cardiac output, and peripheral resistance curves follow the same patterns. Blood chemistry changes reflect volume depletion in both situations. Studies of changes in oxygen partial pressure, carbon dioxide partial pressure, and

---

[39]Kenney PR: Neuroendocrine response to volume loss. Trauma Quarterly 2(3):18-27, 1986.

[40]Crowell JW and Guyton AC: Evidence favoring a cardiac mechanism in irreversible hemorrhagic shock. Am J Physiol 201:893-896, 1961.

[41]Crowell JW and Guyton AC: Further evidence favoring a cardiac mechanism in irreversible hemorrhagic shock. Am J Physiol 203:248-252, 1962.

[42]Hershey SG: Experimental irreversible shock. In Hershey SG (ed): Shock, Boston, Little, Brown Company, c1964.

pH both in hemorrhagic shock (43-44) and in this model show similar results. Though the partial pressures of oxygen obtained were within normal limits, this may not reflect oxygenation of the peripheral tissues. Red cells trapped in the small microscopic vessels are unlikely to contain as much oxygen as the flowing red cells available for blood gas analysis. Here, the main difference between the two models, the progressive rise in the hematocrit and blood viscosity in the pure plasma loss model opposed to a decrease in hemorrhagic shock, may be significant. Although the red cells are important oxygen carriers and buffer system elements, the sparing of the red cell mass in this model may be detrimental, in so far as the increase in hematocrit promotes erythrocyte sludging that may further impair flow in the microcirculation.

CONCLUSION

This model permits the observation of hemodynamic responses when intravascular volume is lost without concomitant decrease in the red cell mass. It mimics the changes occurring in burn trauma, where increased sieving of fluid through the capillary walls to the extravascular space without significant loss of red cell mass is the main event producing the hemodynamic instability of the immediate postburn period. The fact that the filter membranes and pores are unresponsive to biological compensatory mechanisms may be less than ideal but the principal mechanisms governing capillary filtration and volume loss can be studied.

In future work, the addition of a blood pump to the circuit will make possible the study of both the relation of the rate of fluid loss to hemodynamic changes and the resuscitative effects of replacement with various fluids. Better understanding of the peculiarities of this type of hypovolemic shock should allow for more precise fluid resuscitation of burned patients.

PRESENTATIONS/PUBLICATIONS

None.

---

[43] Hartong JM, Dixon RS, and Meyers TT: Use of an *in vivo* oxygen electrode to determine the effect of hemorrhagic shock on liver oxygen tension. Am J Surg 133:607-608, 1977.

[44] Meyers JR, Meyer JS, and Baue AE: Does hemorrhagic shock damage the lung? J Trauma 13:509-519, 1973.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA311488	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY NONE	4. KIND OF SUMMARY A	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISP'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	076
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) Cellular Host Defense Function After Thermal Injury: Assessment by Flow Cytometry of Peripheral Blood Cells

12. SUBJECT AREAS
06 01 Biochemistry 06 05 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 05	CONT	DA	C

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED APPROVED BY [signature]

a. DATE EFFECTIVE	RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
c. TYPE / d. AMOUNT	86	0.1	2
e. KIND OF AWARD / f. CUM/TOTAL	87	0.4	35

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BURLESON, D G
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-7138
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): MASON, A D g. NAME OF ASSOCIATE INVESTIGATOR (If available):

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Flow Cytometry; (U) Lymphocyte Subpopulations; (U) Burn Injury; (U) Infection; (U) Immunocompetence;

23. TECHNICAL OBJECTIVE 24 APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (U) Volunteers; (U) ILIR; (U) RAII

23. (U) Burn injury is a major threat to soldiers on the modern battlefield. Opportunistic infection is a common complication in burned soldiers and is a major source of mortality and morbidity. The objectives of this study are to develop methods to analyze the complex leukocyte mixtures seen in the blood of burn patients, to quantitate the changes in cell morphology and function that occur, and to correlate those findings with the clinical outcome of the patient with the ultimate goal being to develop tests for effective diagnosis of infection susceptibility and evaluation of the effectiveness of new treatment modalities.

24. (U) The development of techniques for evaluation of the immune status of burn patients will focus on the evaluation of lymphocyte subpopulations composition and function. The resolving power of the flow cytometer will be used to differentiate the lymphocyte subpopulations. Five measurements will be made on each cell simultaneously, consisting of two light scatter measurements of physical properties and three subpopulation or functional markers. The multiparameter analysis will be correlated with patient mortality and morbidity and compared to parameters measured on control subjects. Abnormal lymphocyte subset composition or function that correlates with patient outcome will be analyzed in more detail for efficacy as a clinical diagnostic tool and as a probe to determine how the abnormality is related to the defect in host defense.

DD FORM 1498 83 MAR — EDITION OF MAR 60 IS OBSOLETE

CONTINUATION OF DD FORM 1498 FOR "CELLULAR HOST DEFENSE FUNCTION AFTER THERMAL INJURY: ASSESSMENT BY FLOW CYTOMETRY OF PERIPHERAL BLOOD CELLS"

25. (U) 8605 - 8609. Detecting surface markers for relatively small populations has been particularly difficult because of contaminating blood leukocytes. A panel of monoclonal reagents was surveyed to find one marker with specificity to immature granulocytes and monocytes in patient lymphocyte preparations. One reagent was found with a strong specificity against monocyte antigen and a weak affinity for myelocytic cells. A combination of this monoclonal stain and right angle light scatter provided the discrimination necessary to exclude the contaminating cells and improve the analysis of the remaining lymphocytes, which could be stained with fluorescent subset surface markers and/or functional markers. The effectiveness of the new technique was demonstrated by the determination of surface markers of NK cells in heavily contaminated patient samples. Previously, the measurement of the NK cell subpopulation was made on a diffuse population constituting an erroneously high 11 percent of the cells. Using this new technique, NK cell surface markers were found on a precisely defined population that made up six percent of the lymphocyte population.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: CELLULAR HOST DEFENSE FUNCTION AFTER THERMAL INJURY: ASSESSMENT BY FLOW CYTOMETRY OF PERIPHERAL BLOOD CELLS

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

21 April 1986 - 30 September 1986

INVESTIGATORS

David G. Burleson, PhD, Lieutenant Colonel, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: CELLULAR HOST DEFENSE FUNCTION AFTER THERMAL INJURY: ASSESSMENT BY FLOW CYTOMETRY OF PERIPHERAL BLOOD CELLS

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 21 Apr 86 through 30 Sep 86

INVESTIGATORS: David G. Burleson, PhD, Lieutenant Colonel, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

The dramatic increase in abnormal leukocytes found in peripheral blood following burn injury interferes with the accurate measurement of minor lymphocyte subpopulations. We have developed an improved procedure to measure lymphocyte subpopulations in burn patients. This procedure is based on the ability of the monoclonal antibody anti-LeuM3 to bind the nonlymphoid cells which frequently contaminate lymphocyte preparations from burn patients. Natural killer cell subpopulations from seven burn patients were monitored twice weekly for up to five weeks postburn. Lymphocytes were prepared on Ficoll-Hypaque gradients and stained with fluorescein-labeled anti-LeuM3 (monocyte/macrophage marker) and phycoerythrin-labeled anti-Leu11 (natural killer cell marker) simultaneously. The double-labeled cells were analyzed by flow cytometry. Leu11 positive cells made up 16.6 percent of the patient cells taken from the lymphocyte fraction of the Ficoll-Hypaque gradient compared to 13.6 percent in normal controls. When standard light scatter gates were used to restrict the nonlymphoid cells from the analysis, 9.1 percent of the analyzed patient cells bound anti-Leu11 compared to an equivalent number of control cells (9.4 percent). Light scatter gates did not remove all of the nonlymphoid cells from analysis as 8.2 percent of the patient cells and 4.2 percent of the control cells bound anti-LeuM3 after gating. More than half of these contaminating cells also bound anti-Leu11, erroneously increasing the apparent number of natural killer cells present. If the cells that bound LeuM3 were also removed from analysis (gated), 5.2 percent of the remaining patient cells bound anti-Leu11 compared to 7.0 percent for control ($P < 0.05$). The presence of contaminating nonlymphoid cells masked the proportionate decrease in natural killer cells that occurred in the patient population. Anti-LeuM3 used in

addition to light scatter as a gating parameter provides the discrimination necessary to exclude contaminating cells and allow more accurate analysis of the remaining lymphocytes.

CELLULAR HOST DEFENSE FUNCTION AFTER THERMAL INJURY: ASSESSMENT BY FLOW CYTOMETRY OF PERIPHERAL BLOOD CELLS

INTRODUCTION

Analysis of the status of immunity in burn patients is hindered by the fact that standard techniques for preparing peripheral blood lymphocytes from burn patients produce preparations that are heavily contaminated with nonlymphoid cells. Flow cytometry is currently the most sophisticated technique for resolving complex mixtures of white blood cells so that the composition of lymphocyte subpopulations can be analyzed. Standard flow cytometry techniques define populations of peripheral blood leukocytes using measurement of light scatter intensity (1). These techniques are adequate for analyzing cells from patients with relatively normal leukocyte morphology. However, lymphocyte preparations obtained from burn patients are too complex to be resolved sufficiently by light scatter techniques for accurate analysis (2-3).

A new technique has been developed to help discriminate between types of peripheral blood leukocytes from burn patients. This technique uses a monoclonal antibody that binds nonlymphoid cells to resolve the lymphocyte populations from other nonlymphoid populations. This new technique has used to measure the natural killer cell population in burn patients. The increased accuracy provided by this technique revealed that the level of natural killer cells in burn patients was decreased compared to unburned controls. This decrease was masked by contaminating cells when standard light scatter techniques were employed to measure natural killer cells.

MATERIALS AND METHODS

Cell Preparation. Patient samples were collected in Vacutainer™ tubes containing ethylenediaminetetra-acetic acid (Becton-Dickinson #6450, Becton, Dickinson and Company, Rutherford, New Jersey). Blood was diluted 1:2 and layered

---

[1]Salzman GC: Light scattering analysis of single cells. In Catsimpoolas N (ed). Cell Analysis, Volume 1. New York: Plenum Press, c1982, pp 111-143.

[2]Davis CF, Roderick ML, Wood JJ, et al: Effect of separation techniques for human peripheral blood cells on parameters of immune response and inflammation (Abstract 4536). Fed Proc 44:1186, 1985.

[3]Calvano SE, Reid AMM, de Riesthal HF, et al: Granulocyte contamination of Ficoll-Hypaque preparations of mononuclear cells following thermal injury (Abstract 48). Proceedings of the American Burn Association, 17th Annual Meeting, 1985.

onto Ficoll-Paque™ (Pharmacia Fine Chemical Company, Uppsala, Sweden) in 15-milliliter plastic conical centrifuge tubes (Corning #25310, Corning Glass Works, Midfield, Massachusetts). The gradients of diluted whole blood on Ficoll-Hypaque were centrifuged at 450 times gravity for 30 minutes. Cells obtained from the interface layer were washed three times in Hank's balanced salt solution (HBSS) and resuspended at an appropriate cell concentration for staining with fluorescent monoclonal antibodies. Contaminating red blood cells were lysed in ammonium chloride buffer (4.15 grams ammonium chloride, 0.5 gram potassium bicarbonate, and 0.185 gram ethylenediaminetetra-acetic acid in 500 milliliters water, pH = 7.3). The remaining leukocyte mixture was centrifuged through 0.8 milliliters fetal calf serum to remove extraneous debris and reconstituted at 1×10^7 cells per milliliter.

Staining with Monoclonal Antibodies. Fluorescein isothiocyanate-labeled anti-LeuM3 (Becton, Dickinson and Company), which has a high specificity for monocytes and a weak affinity for granulocytes (4-5), was used as the first marker and phycoerythrin-labeled anti-Leu11 (Becton, Dickinson and Company), which binds to a population of cells with a high level of natural killer cell activity (6-8), was used as the second surface marker. The monoclonal antibodies were diluted 1:5 with 25 microliters HBSS and added to 25 microliters of the cell suspension containing 2.5×10^6 cells. Cells were incubated with the anti-LeuM3 for 15 minutes, washed twice, and reconstituted in 50 microliters HBSS and 25 microliters of anti-Leu11 (diluted 1:5) was added. After a 15-minute

---

[4]Dimitriu-Bona A, Burmester GR, Waters SJ, et al: Human mononuclear phagocyte differentiation antigens. I. Patterns of antigenic expression on the surface of human monocytes and macrophages defined by monoclonal antibodies. J Immunol 130:145-152, 1983.

[5]Herrmann F, Komischke B, Odenwald E, et al: Use of monoclonal antibodies as a diagnostic tool in human leukemia. I. Acute myeloid leukemia and acute phase of chronic myeloid leukemia. Blut 47:157-163, 1983.

[6]Phillips JH and Babcock GF: NKP-15: A monoclonal antibody against purified human natural killer cells and granulocytes. Immunol Letters 6:143-149, 1983.

[7]Phillips JH, Le AM, and Lanier LL: Natural killer cells activated in a human mixed lymphocyte response culture identified by expression of Leu-11 and Class II histocompatibility antigens. J Exp Med 159:993-1008, 1984.

[8]Lanier LL, Le AM, Phillips JH, et al: Subpopulations of human natural killer cells defined by expression of the Leu7 (HNK-1) and Leu11 (NK-15) antigens. J Immunol 131:1789-1796, 1983.

incubation with the second antibody, the cells were washed twice and fixed in one-percent paraformaldehyde in HBSS.

Flow Cytometry. Cells were analyzed on a Becton-Dickinson Model 400 flow cytometer modified to include an additional photomultiplier, data channel, and interface to a consort 40 data analysis system. An EPICS model 753 (Coulter Corporation, EPICS Division, Hialeah, Florida) interfaced to an EASY88 data analysis system was also used. Instruments were calibrated at the beginning of each run with beads of known fluorescein content. The positive cutoff for each fluorescent marker was determined on an isotypic control using appropriately stained mouse immunoglobulin of the same isotype as anti-LeuM3 and anti-Leu11. The intensity channel defining the upper two percent of the isotypic control cells was selected as the positive cutoff.

Statistical Analysis. Statistical analysis included a comparison of means between burned and control groups using the student's t-test and was performed on a VAX 11/780 (Digital Equipment Corporation, Maynard, Massachusetts) using BMDP statistical software (Program P7D, BMDP Statistical Software, Inc., Los Angeles, California). Comparison of means before and after gating was performed by Wilcoxon's matched-pairs signed rank's test.

RESULTS

Anti-LeuM3 bound to many of the nonlymphoid cells. The specificity of the reagent for nonlymphoid burn patient cells separated on Ficoll-Hypaque gradients was confirmed by sorting fluorescently-stained cells on the flow cytometer. The positive and negative cells were sorted into separate containers and used make slides for differentiation by light microscopy after staining with Wright's stain. Within the limits of sorting purity (90 to 95 percent) LeuM3 positive cells were exclusively monocytes and granulocytes in various stages of maturity and LeuM3 negative cells were lymphocytes.

Cells stained with LeuM3 and Leu11 were analyzed by flow cytometry. The potential impact of the contaminating cells on the determination of lymphocyte subpopulations can be surmised after examining Figure 1. This figure is a comparison of an isometric representation of the phycoerythrin staining intensity of Ficoll Hypaque-purified cells from a burn patient with a 44-percent total body surface area burn on postburn day six and the corresponding preparation from an unburned control. The FALS intensity is displayed on the X axis, phycoerythrin-labeled Leu11 fluorescence intensity on the Y axis, and the cell number on the Z axis. These figures demonstrate that a large number of cells in the mixture of cells from the patient bind Leu11. Many of the positive cells

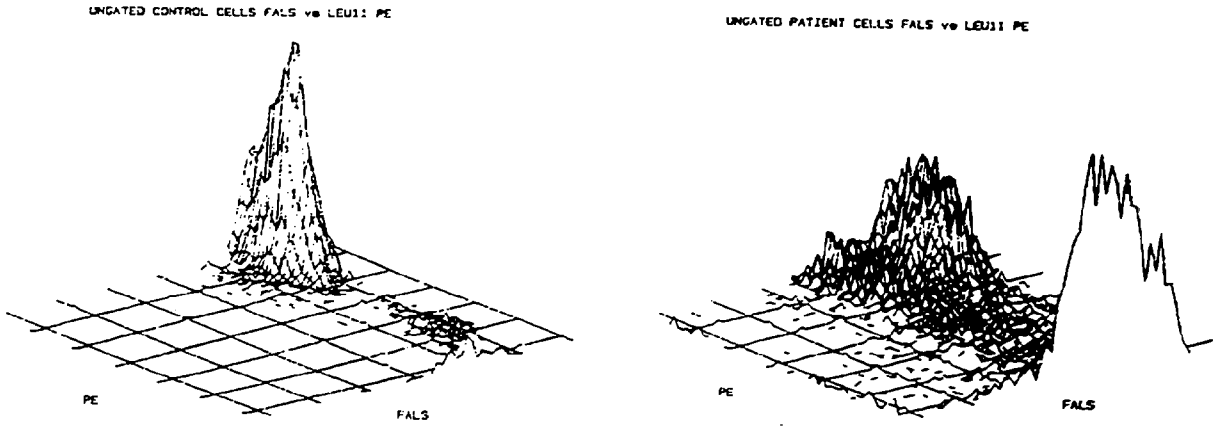


FIGURE 1. A Comparison of Leu11 positives in a burn patient and control. The cell distribution from a patient with a 44-percent total body surface area burn and an unburned control are represented in an isometric histogram. Intensity of forward angle light scatter is represented on the X axis, phycoerythrion intensity on the Y axis, and the cell number on the Z axis.

are nonlymphoid cells binding anti-Leu11 nonspecifically. The interference from the autofluoresence and nonspecific binding of monocytes contribute a background fluorescence level that is unacceptable even in Ficoll-Hypaque preparations from normal individuals. Standard flow cytometry techniques routinely restrict the nonlymphoid cells from the analysis on the basis of light scatter intensity measurements. Those cells whose forward and side scatter light intensities are not characteristic of lymphocytes are not included in the analysis of fluorescence intensity. This technique is referred to as light scatter gating. The procedure works well for those lymphocyte studies where nonlymphoid contamination is minor compared to the number of lymphocytes present. Light scatter intensity is derived principally from morphological characteristics of the cells. Since monocytes can be morphologically similar to lymphocytes, the resolution of lymphocytes and monocytes is not large. Forward and right angle light scatter patterns for the patient and control depicted in Figure 1 are compared in Figure 2. Forward angle light scatter is shown on the X axis, right angle light scatter is shown on the Y axis, and the cell number is shown on the Z axis. The areas characteristic of lymphocytes, monocytes, and granulocytes are indicated on the figure. If gates are set so that only the cells whose light scatter patterns fall within the lymphocyte area are analyzed, the number of cells positive for Leu11 is greatly reduced in both patient and control samples (Figure 3). However, there is still an unacceptably large number of Leu11 positive nonlymphoid cells in the burn patient preparation which also bind anti-LeuM3. These double positive cells can be eliminated from the determination of anti-Leu11 positives if restrictive gates are established that eliminate the LeuM3 positive cells in addition to those eliminated on the basis of light scatter intensity. Analysis of the isometric histogram in Figure 4 reveals that the intensity of staining of anti-LeuM3 on patient cells is such that monocytes can easily be differentiated from lymphocytes. Although they stain with less intensity, cells of granulocytic origin also bind a sufficient amount of stain to be differentiated from the lymphocytes. When patient cells are analyzed using the LeuM3 gate in addition to light scatter gates, the resolution is greatly improved as shown in Figure 5. The cell preparation in Figure 5A is the same as that in Figure 3A which depicted cells prior to LeuM3 gating.

Ficoll-Hypaque-purified cells from seven burn patients were monitored twice weekly for up to seven weeks postburn (five samples). Cells were simultaneously stained for LeuM3 and Leu11 and the results were obtained after, no gating, light scatter gating, and combined light scatter and LeuM3 gating. Ten healthy controls were also monitored during this period an average of two times (20 samples). The effect of gating on the number of cells analyzed and the number of LeuM3 positive cells

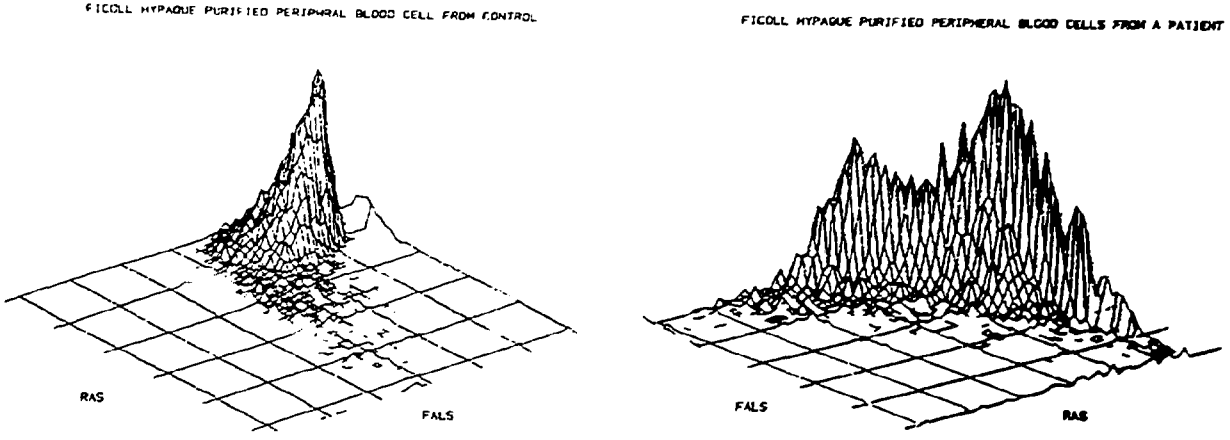


FIGURE 2. A comparison of light scatter histograms between a burn patient and unburned control. This is an isometric histogram of forward angle light scatter and right angle scatter containing the same cells shown in Figure 1.

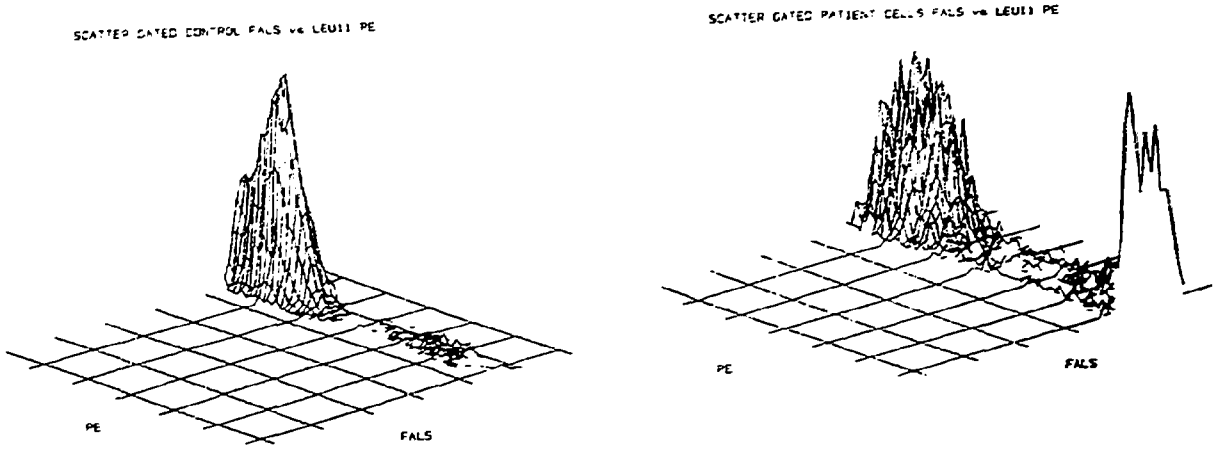


FIGURE 3. Relative distributions of Leu11 and LeuM3 positives after light scatter gating of burn patient cells. This isometric histogram of the same patient from the above figures displays relative fluorescence intensity for fluorescein isothiocyanate-labeled LeuM3 on the X axis, phycoerythrin-labeled Leu11 on the Y axis, and the cell number on the Z axis. The four combinations of negative and positive cells are noted on the figure.

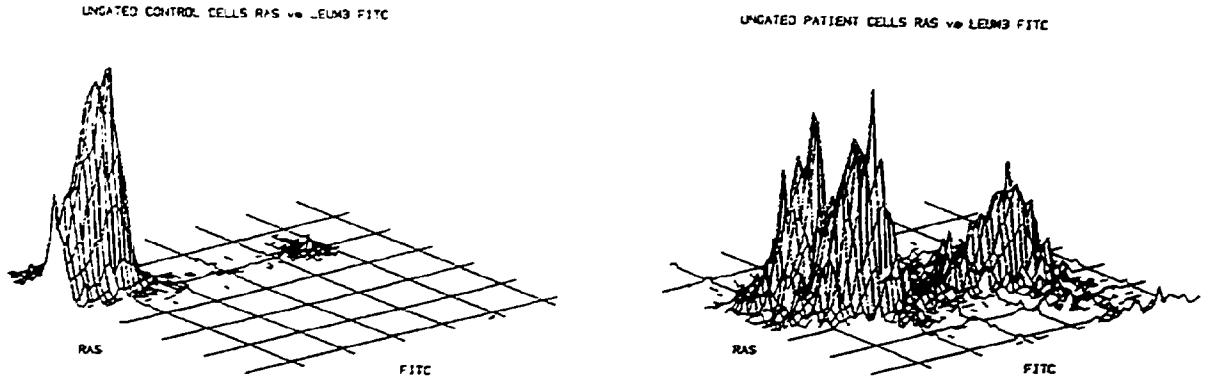


FIGURE 4. Relative intensity of anti-LeuM3 binding of a lymphocyte preparation from a burn patient. The relative intensity of fluorescein isothiocyanate fluorescence for lymphocytes, monocytes, and granulocytes is shown on the Y axis versus right angle scatter on the X axis. The relative staining of the three cell types is noted on the figure.

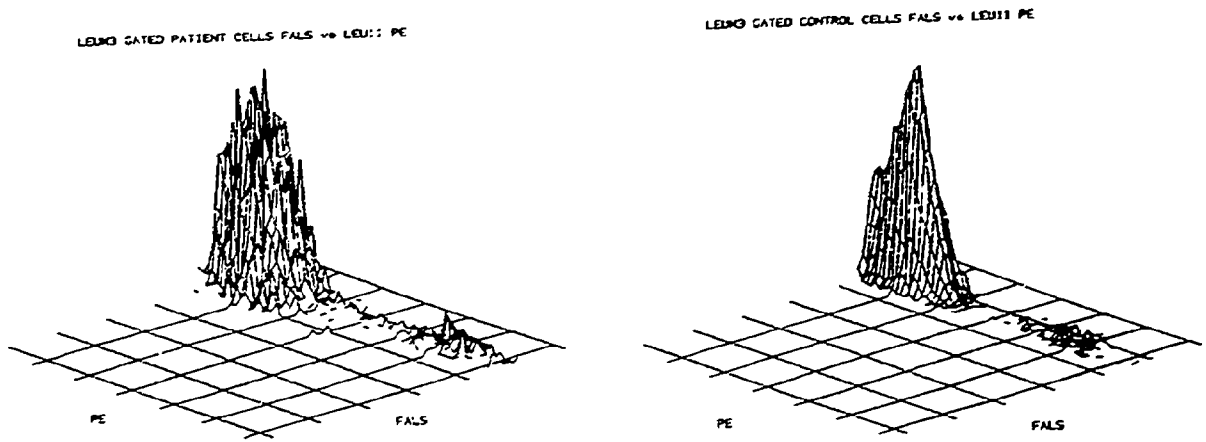


FIGURE 5. Comparison of Leu11 positives obtained before and after anti-LeuM3 gating. These histograms represent the distribution from the same cell preparation as Figure 1B after light scatter gating and after light scatter gating with additional anti-LeuM3 gating.

before and after light scatter gating are compared in Table 1. Light scatter gating removed a large number of cells from the analysis, but there were an average of 8.4 percent of the patient cells and 4.2 percent of the control cells that were positive for LeuM3. The results of the combination of light scatter gating and LeuM3 gating on the determination of the natural killer cell population are shown in Table 2. The contaminating nonlymphoid cells remaining after light scatter gating obscured a significant decrease in the natural killer cells in the burned patient population. A comparison of each patient individually against the control population showed that three of seven patients had a significant decrease in the natural killer cell population when LeuM3 gating was used compared to one of seven when only light scatter gating was used.

DISCUSSION

In order for lymphocyte function and cell surface antigen measurements to be interpretable, the leukocyte composition of the cell population being measured must be known. A perusal of the literature in burn immunology for the last 10 years shows very little morphological confirmation of the purity of the lymphocytes used for the reported experiments. Recently, several laboratories have acknowledged that the problem of cell contamination exists in populations of Ficoll-Hypaque-purified cells from burn patients (2-3). The analytical power of the flow cytometer and it's ability to analyze cells individually rather than collectively make it the instrument of choice with which to make measurements of surface antigens or functional parameters of complex mixtures of leukocytes.

Natural killer cells are a relatively small heterogeneous group of peripheral blood cells with the morphology of large granular lymphocytes. They have the ability to spontaneously lyse certain malignant cell lines and virus-infected cells *in vitro*. Their exact *in vivo* function is still uncertain, but tumor rejection and defense against viral infections have been most frequently postulated (9-10).

---

[9]Bancroft GJ, Shellam GR, and Chalmer JE: Genetic influences on the augmentation of natural killer (NK) cells during murine cytomegalovirus infection: correlation with patterns of resistance. J Immunol 126:988-994, 1981.

[10]Steinhauer EH, Doyle AT, Reed J, *et al*: Defective natural cytotoxicity in patients with cancer: normal number of effector cells but decreased recycling capacity in patients with advanced disease. J Immunol 129:2255-2259, 1982.

TABLE 1

EFFECT OF GATING ON AND LeuM3 POSITIVES

	No Gate	Light Scatter Gate	LeuM3 Gate
Patient Group			
Cells Analyzed	10,000	5,574 ± 1,466	5,470 ± 1,586
LeuM3 Positive	18.52% ± 9.8%	8.42% ± 3.97%	0
Control Group			
Cells Analyzed	10,000	8,056 ± 1,205	7,693 ± 1,565
LeuM3 Positive	12.96% ± 6.3%	4.24% ± 3.1%	0

Data was collected on 10,000 cells for two light scatter parameters and two fluorescent probes. Cells were first gated on forward angle light scatter and right angle scatter and then were further gated on fluorescein isothiocyanate-labeled LeuM3. The LeuM3 positives were determined for each of the first two gates. The mean for burn patients (n = 51) and control patients (n = 20) are shown as ± standard deviation.

TABLE 2

COMPARISON OF LeuM3 GATING AND LIGHT SCATTER GATING ON Leu11 POSITIVES

	No Gate	Scatter Gate	LeuM3 Gate	Significance
Control Group	17.8 ± 6.8	10.04 ± 4.44	5.44 ± 2.82	P < 0.01
Patient Group	16.1 ± 5.8	9.59 ± 3.88	7.30 ± 3.88	P < 0.01
Significance	NS	NS	P < 0.05	

The number of Leu11 positives were determined after a combination of LeuM3 gating and light scatter gating. The mean average standard deviation of Leu11 positives are shown for burn patients (n = 51) and control patients (n = 20).

Stein et al (11) recently reported that natural killer activity was decreased in lymphoid cells from burn patients compared to unburned controls. Anti-Leu11 binding and the methods of gating the patients cells were not reported. However, the proportion of cells positive for Leu7, a monoclonal antibody recognizing large granular lymphocytes, was reported to be variable and not correlated with the decrease in natural killer activity.

Many functional assays such as phytohemagglutinin stimulation of lymphocyte proliferation on using cells from burn patients have yielded equivocal results (12-30). This may be due to the complex cell populations used in the experiments.

---

[11]Stein MD, Gamble DN, Klimpel KD, et al: Natural killer cell defects resulting from thermal injury. Cell Immunol 86:552-556, 1984.

[12]Antonacci AC, Good RA, and Gupta S: T-cell subpopulations following thermal injury. Surg Gynecol Obstet 155:1-8, 1982.

[13]Miller CL and Baker CC: Changes in lymphocyte activity after thermal injury: the role of suppressor cells. J Clin Invest 63:202-210, 1979.

[14]Daniels JC, Sakai H, Cobb EK, et al: Evaluation of lymphocyte reactivity studies in patients with thermal burns. J Trauma 11:595-601, 1971.

[15]Mahler D and Batchelor JR: Phytohaemagglutinin transformation of lymphocytes in burned patients. Transplantation 12:409-411, 1971.

[16]Keane RM, Munster AM, Birmingham W, et al: Suppressor cell activity after major injury: indirect and direct functional assays. J Trauma 22:770-773, 1982.

[17]Munster AM, Eurenius K, Katz RM, et al: Cell-mediated immunity after thermal injury. Ann Surg 177:139-43, 1973.

[18]Wolfe JHN, Saporoschetz I, Young AE, et al: Suppressive serum, suppressor lymphocytes, and death from burns. Ann Surg 193:513-520, 1981.

[19]Campa M, Benedettini G, De Libero G, et al: The suppressive activity of T-lymphocytes and serum factors in burned patients. Burns 8:231-237, 1981.

[20]Baker CC, Trunkey DD, and Baker WJ: A simple method of predicting severe sepsis in burn patients. Am J Surg 139:513-517, 1980.

[21]Baker CC, Miller CL, and Trunkey DD: Predicting fatal sepsis in burn patients. J Trauma 19:641-648, 1979.

[22]Antia NH, Srinivasan R, Mistry N, et al: The treatment of burns: immunological studies in burns. Burns 4:55-60, 1977.

[23]Ischizawa S, Sakai H, Sarles HE, et al: Effect of thymosin on T-lymphocyte functions in patients with acute thermal burns. J Trauma 18:48-52, 1978.

Tritiated thymidine incorporation of cell mixtures of unknown composition are likely to be unpredictable and the results therefore uninterpretable. Specific gating techniques like the one described in this report could be used to accurately define which subpopulations respond to specific or nonspecific stimulants and provide meaningful data from studies of the functionality of lymphocytes from burn patients.

PRESENTATIONS/PUBLICATIONS

Burleson DG: Quality control in clinical flow cytometry. Presented to the 11th Annual Meeting of the Society of Armed Forces Laboratory Scientists, San Antonio, Texas, 16-20 March 1986.

---

[24]Baker CC, Miller CL, Trunkey DD, et al: Indentity of mononuclear cells which compromise the resistance of trauma patients. J Surg Res 26:478-487, 1979.

[25]Eurenius K and Mortensen RF: The phytohemagglutinin (PHA) response in the thermally injured rat. Int Arch Allergy Appl Immunol 40:707-718, 1971.

[26]Munster AM, Winchurch RA, Birmingham WJ, et al: Longitudinal assay of lymphocyte responsiveness in patients with major burns. Ann Surg 192:772-775, 1980.

[27]Wolfe JHN, Wu AVO, O'Connor NE, et al: Anergy, immunosuppressive serum, and impaired lymphocyte blastogenesis in burn patients. Arch Surg 117:1266-1271, 1982.

[28]Hansbrough J, Peterson V, Zapata-Sirvent R, et al: Postburn immunosuppression in an animal model. II. Restoration of cell-mediated immunity by immunomodulating drugs. Surgery 95:290-295, 1984.

[29]O'Mahony JB, Palder SB, Wood JJ, et al: Depression of cellular immunity after multiple trauma in the absence of sepsis. J Trauma 24:869-875, 1984.

[30]Faist E, Kupper TS, Baker CC, et al: Depression of cellular immunity after major injury. Arch Surg 121:1000-1005, 1986.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA311489	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	077
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) A Study of Biochemical Changes in the Cellular Environment of Tissue of the in vivo Partial-Thickness Rat Burn Wound

12. SUBJECT AREAS
06 01 Biochemistry 06 16 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 09	CONT	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY Basil M. Pruitt

a. DATE EFFECTIVE		c. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
c. TYPE	d. AMOUNT	86	0.1	3
e. KIND OF AWARD	f. CUM/TOTAL	87	0.7	30

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (include zip code) Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR BROWN, W L
d. TELEPHONE NUMBER (include area code) 512-221-2720	d. TELEPHONE NUMBER (include area code) 512-221-4652
21. GENERAL USE FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (if available) MASON, A D g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burn Wound Metabolism; (U) Edema; (U) Burn Injury; (U) Lab Animals; (U) Rats; (U) ILIR; (U) RAII

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

23. (U) The purpose of this study is to determine biochemical and metabolic changes that occur in the in vivo partial-thickness rat burn wound during the early postburn period which might be deleterious to recovery of function in cells in which thermal injury was potentially reversible. Cellular injury due to short episodes of ischemia in heart, liver, muscle, and brain is reversible but becomes irreversible when ischemia and local tissue acidosis is prolonged. If this same sequence of events is found to occur in the in vivo rat burn wound, it may be possible to block or reverse the metabolic changes and limit the progression in the size and degree of injury in wounds of burned soldiers.

24. (U) Microelectrodes will be used to measure changes in extracellular potassium ion content and in pH and/or carbon dioxide partial pressure at various sites in the in vivo burn wound. Samples from sites adjacent to the microelectrodes will be taken to measure selected metabolites using enzymatic methods. Cells and subcellular organelles will be isolated by centrifugation in self-generating gradients or Percoll for measurement of changes in cellular function with time postburn.

25. (U) 8510 - 8609. This is a new project. Orders for equipment and supplies required to begin the study have been placed.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE. U.S. G.P.O. 1984-421-646/12001

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA305253	2. DATE OF SUMMARY 86 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 85 10 01	4. KIND OF SUMMARY K	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISP'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	078
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE *(Precede with Security Classification Code)* (U) Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model

12. SUBJECT AREAS
06 01 Biochemistry 06 13 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
84 09	86 09	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Basil A. Pruitt
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
86	1.0	39
87	0.0	0

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS *(include zip code)* Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR SHIPPEE, R L
d. TELEPHONE NUMBER *(include area code)* 512-221-2720	d. TELEPHONE NUMBER *(include area code)* 512-221-7138
21. GENERAL USE FINA MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR *(if available)* WILSON, S W g. NAME OF ASSOCIATE INVESTIGATOR *(if available)* KING, N L

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Thermal Injury; (U) Zinc Homeostasis; (U) Immunocompetence; (U) Lab Animals; (U) Rats; (U) ILIR; (U) RAII

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23. (U) The main objective of this research is to determine the changes in zinc metabolism caused by burn and infection in a murine model. The experiments are designed to study the effect of injury on physiological control mechanisms at the whole body, organ, and molecular levels. Information obtained form these studies will provide a better understanding of the postinjury changes in the metabolism of this important trace element and the role of these changes in septic complications, ultimately leading to the development of optimal supplementation of zinc in burned humans.

24. (U) To determine endogenous fecal and urine losses after burn injury, a semi-purified zinc deficient (> 0.5 ppm) diet is fed to rats. The zinc supplemented groups are given a daily subcutaneous zinc injection (one milligram/kilogram body weight). Total fecal and urine are collected for 10 days after the rats are given a 30-percent full-thickness burn. After receiving the burn injury, the burn group is divided up into a zinc-supplemented and a zinc-nonsupplemented groups. Three nonburned control groups are used, i.e., zinc sufficient/ad libitum fed, zinc sufficient/pair fed, and zinc deficient. Ten days postburn, the rats are sacrificed and peripheral blood lymphocytes isolated and stained using fluorecein labeled monoclonal antibodies. Flow cytometry is used to identify total T-cells, T-helper cells, and T-suppresser cells.

DD FORM 1498 83 MAR EDITION OF MAR 68 IS OBSOLETE

CONTINUATION OF DD FORM 1498 FOR "PRELIMINARY STUDIES OF ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL"

25. (U) 8510 - 8609. No significant increases in endogenous fecal and urine excretion were caused by the burn injury. The effect of burn and burn plus zinc deficiency caused a decrease in total peripheral blood T-cells. However, the burn/zinc deficient rats showed a decrease in T-helper cell populations and an increase in T-suppresser cell populations while the burn/zinc sufficient animals were comparable with the control/zinc sufficient and control/zinc deficient rats. Experiments are in the process of being completed that will assess the effect of a 60-percent full-thickness burn on zinc homeostatic control and changes in T-cell and T-cell subpopulation distribution. Project transferred to DA311499.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Ronald L. Shippee, PhD, Captain, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: PRELIMINARY STUDIES ON ZINC HEMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: Ronald L. Shippee, PhD, Captain, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Nonlethal 30-percent total body surface area scald burns administered to rats maintained on sufficient and deficient zinc intakes did not place the rats in additional zinc deficit relative to the appropriate control groups when obligatory fecal and urinary zinc losses and circulating plasma zinc concentrations were used as assessment criteria. However, a significant ($P < 0.05$) increase in circulating blood T-suppressor lymphocytes occurred in burned animals maintained on a zinc-deficient regimen during a 10-day period postburn. Although this suggests an interrelationship between immunocompetence, burn injury, and zinc nutriture, it remains to be shown how these changes relate to infection or mortality associated with burn injuries.

PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL

INTRODUCTION

Subnormal serum zinc concentrations have been shown to be part of the physiological response to burn injury in humans (1-3). Davies and Fell (4) reported that zinc is excreted in the urine of patients with 10 to 30-percent total body surface area (TBSA) burns at twice the rate observed in normal individuals; excretion rates approaching five times normal rates were observed in patients with more extensive burns (30 to 75 percent of the TBSA). It has been suggested that enteral supplementation of 10 times the recommended daily zinc normal requirement may be necessary to meet the needs of burn patients (5).

There is a paucity of literature concerning the use of animal models to study zinc metabolism after burn injury. Oh et al (6) have reported the effect of various stresses, including burn injury, on accumulation of zinc bound to metallothionein in the liver and kidneys of weanling male Long-Evans rats. Cold, exercise, and the injection of CCl_4 caused a marked increase in radioactive zinc accumulation in metallothionein from liver cytosol when compared to nonstressed controls, while burn injury of about one percent of the TBSA caused only a slight increase over controls (6).

Although clinical observations in burned patients and controlled animal studies indicate that thermal injury causes disturbances in zinc metabolism, very little is known about the homeostatic mechanisms involved in the control of zinc

---

[1]Nielsen SP and Jemec B: Zinc metabolism in patients with severe burns. Scand J Plast Reconstrs Surg 2:47-52, 1968.

[2]Cohen IK, Schechter PJ, and Henkin RI: Hypogeusia, anorexia, and altered zinc metabolism following thermal burn. JAMA 223:914-916, 1973.

[3]Sanchez-Agreda M, Cimorra GA, Mariona M, et al: Trace elements in burned patients: studies of zinc, copper and iron contents in serum. Burns 4:28-31, 1977.

[4]Davies JW and Fell GS: Tissue catabolism in patients with burns. Clin Chim Acta 51:83-92, 1974.

[5]Sandstead HH: Nutrition in trauma and burns. In Dialogues in Nutrition. Margie JD (ed). Bloomfield Health Learning Systems, Inc., Volume 2, 1979, p 4.

[6]Oh SH, Deagen JT, Whanger PD, et al: Biological function of metallothionein. V. Its induction in rats by various stresses. Am J Physiol 234:E282-E285, 1978.

nutriture after burn injury. Powanda et al (7) have reported the effect of a much larger burn (30 percent of the TBSA) on redistribution of zinc in male albino rats. That study also included the complication of infection. To obviate differences in food intake caused by burning or infection, rats were fasted following burning and infection with Pseudomonas aeruginosa. Control-fasted rats had a gradual decrease in serum zinc with no accumulation of zinc in the liver over the six days of the study. The serum concentrations of zinc in both burn-fasted (BF) and burn-fasted-infected (BFI) rats decreased sharply 24 hours after thermal injury, while levels of zinc in the liver increased. During the next five days serum zinc concentrations decreased further in BFI rats and remained constant in BF rats, while levels of liver zinc increased with time in BFI rats and decreased in BF rats.

This report describes the results of preliminary studies of zinc metabolism in a murine burn model (8). Because excretion into the intestinal lumen has been shown to be a quantitatively important homeostatic control mechanism of zinc metabolism (9), the present study was conducted to determine the effect of a full-thickness burn of 30 percent of the TBSA on intestinal endogenous excretion of zinc in the burned rat. Standard gel column techniques were used to determine changes in protein bound zinc in hepatic and intestinal mucosal cytosol preparations.

We also investigated possible interactions between burn injury, zinc nutriture, and cellular immunocompetence. There exists a considerable amount of evidence to support an interrelationship between zinc nutriture and alterations in

---

[7]Powanda MC, Villarreal Y, Rodriguez E Jr, et al: Redistribution of zinc within burned and burned infected rats. Proc Soc Exp Biol Med 163:296-301, 1980.

[8]Walker HL and Mason AD Jr: A standard animal burn. J Trauma 8:1049-1051, 1968.

[9]Weigand E and Kirchgessner M: Homeostatic adjustments in zinc digestion to widely varying dietary zinc intake. Nutr Metab 22:101-112, 1978.

cellular immune processes (10-13). Based on this knowledge and the well established immunological consequences of burn injury, we investigated the relationship between circulating blood T-lymphocyte populations and burn and/or zinc restriction.

MATERIALS AND METHODS

Housing, Feeding, and Experimental Design. Sixty male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Houston, Texas) weighing ± 350 grams (g) were used in these studies. Rats were housed in individual stainless steel cages, given distilled deionized water ad libitum, and maintained on a 12-hour on, 12-hour off light schedule. The rats were fed a semi-purfied diet (Ziegler, Inc., Post Office Box 95, Gardners, Pennsylvania 17324) designed to meet all the nutrient requirements of the adult rat except for zinc (Table 1). All the rats were fed this zinc deficient diet (< 0.5 parts per million) ad libitum for two weeks and given a daily subcutaneous injection of one milligram (mg) zinc per kilogram body weight (Zn/kgBW) as zinc sulfate. After the two-week equilibration period, rats were weighed and assigned to one of the following regimens in a manner that equalized mean body weight among the treatment groups (n = 12):

Burn sufficient (BS). Administered a 30-perent TBSA burn, injected daily with one mg Zn/kgBW, and fed ad libitum.

Burn deficient (BD). Administered a 30-perent TBSA burn, injected daily with saline, and fed ad libitum.

Control sufficient (CS). Injected daily with one mg Zn/kgBW, and fed ad libitum.

Control deficient (CD). Injected daily with saline and fed ad libitum.

---

[10]Fraker PJ, Hass SM, and Luecke RW: Effect of zinc deficiency on the immune response of the young adult A/J mouse. J Nutr 107:1889-1895, 1977.

[11]Good RA and Fernandes G: Nutrition, immunity, and cancer - a review. Part I: Influence of protein or protein - calorie malnutrition and zinc deficiency on immunity. Clin Bull 9:3-12, 1979.

[12]Oleske JM, Westphal ML, Shore S, et al: Zinc therapy of depressed cellular immunity in acrodermatitis enteropathica. Its correction. Am J Dis Child 133:915-918, 1979.

[13]Dardenne M, Savino W, Berrih S, et al: A zinc-dependent epitope on the molecule of thymulin, a thymic hormone. Proc Natl Acad Sci USA 82:7035-7038, 1985.

TABLE 1

DIET COMPOSITION

Ingredient	Percentage of Diet
Corn Starch	31.2
Sucrose	31.0
Egg White Solids	20.0
Corn Oil	10.0
Cellulose Powder	3.0
Mineral Premix[a]	4.0
Vitamin Premix[b]	0.5
Choline Bitartrate	0.3

[a]International Unit Per Kilogram of Diet: Retinyl palmitate = 30, ergocalciferol = 4, α-tocopherol = 0.05.
Milligram Per Kilogram of Diet: Niacin = 98, pantothenic acid as calcium pentothenate = 55, riboflavin = 20, thiamine as thiamine mononitrate = 18.4, pyroxidine = 8.2, menadione = 2.047, biotin = 1.0, folic acid = 0.5, cyanocobalamin = 0.2.
Grams Per Kilogram of Diet: Inositol = 400.
[b]Grams Per Kilogram of Diet: Ca = 8.997; P = 7.271; Mg = 0.603; K = 2.674 as $CCaO_3$, $CaHO_4P$, MgO, HK_2O_4P, and K_2P_4S; NaCl = 1.224.
Milligrams Per Kilogram of Diet: Cu = 7.42; Fe = 50.89; Mn = 101.30; I = 0.31 as $Cu_2C_6J_4O_7$, $FeC_6H_5O_7$, $Mn_3(C_6H_5O_7)_2$, and KI; citric acid = 90.80.

Control pair-fed (CP). Injected daily with one mg Zn/kgBW and fed the amount of feed eaten in the previous 24-hour period by the burn deficient, weight-paired rats.

Six rats from each group were placed in stainless steel metabolic cages to facilitate collection of fecal and urine excretion from the day the burn-injured rats were burned until 10 days postburn when all rats were sacrificed.

Feces, Urine, Plasma, and Liver Zinc Analysis. Zinc concentrations in fecal excreta were determined by heating at 600° C for three hours, then 1200° C for eight hours. The dry ash was then solubilized in concentrated nitric acid, diluted

to 25 milliliters (ml) with distilled deionized water, and aspirated into an atomic absorption spectrophotometer (Model 5000, Perkin-Elmer, Norwalk, Connecticut 06856).

Zinc and copper concentrations in liver tissue were determined by first perfusing the liver with physiological saline, then removing approximately 700 mg of liver tissue that was dried at 80° C for 12 hours in a vacuum oven, after which 10 ml of an acid solution containing 21.5 percent perchloric acid, 7.0 perent sulfuric acid, and 71.5 percent nitric acid was added. After concentrating on a hot plate to approximately two ml, the wet-ashed tissue was diluted to 25 ml with distilled deionized water and aspirated directly into the atomic absorption spectrophotometer for determination of zinc and copper concentration. The accuracy of the dry and wet ash procedures was confirmed using a certified reference standard (National Bureau of Standards Standard Reference Material, Bovine Liver #1577a, Office of Standard Reference Material, Washington, DC 20234).

Urine samples were aspirated directly into the atomic absorption spectrophotometer for zinc analysis.

Approximately 12 cubic centimeters of heparinized blood was drawn from the inferior vena cava. One ml of a 20 percent trichloroacetic acid solution was added to one ml of whole blood. This solution was vortexed and then centrifuged for 20 minutes at 500 timess gravity. Zinc and copper content of the supernatant was determined by atomic absorption spectrophotometry. The accuracy of this procedure was confirmed by the method of additions using zinc sulfate.

Liver and Intestinal Cytosol Preparation and Analysis. Hepatic and intestinal mucosal cell cytoplasmic zinc binding proteins were characterized using procedures similar to those described by Richards and Cousins (14-15). Single lobes of the perfused livers of each rat within the treatment groups were pooled in a glass-teflon homogenizer. Chilled (4° C) buffer containing 0.25 molar sucrose, 0.9 percent sodium chloride, 0.02 percent sodium azide, and 10 millimolars TRIS-HCl (pH = 8.6) was added at a 2:1 weight/volume ratio. After homogenization, the tissue was centrifuged at 42,000 times gravity (X g) for 30 minutes. The supernatant was pipetted into clean tubes and centrifuged at 42,000 X g for two hours.

---

[14]Richards MP and Cousins RJ: Influence of parenteral zinc and actinomycin D on tissue zinc uptake and the synthesis of a zinc-binding protein. Bioinorg Chem 4:215-224, 1975.

[15]Richards MP and Cousins RJ: Mammalian zinc homeostasis: requirement for RNA and metallothionein synthesis. Biochem Biophys Res Commun 64:1215-1223, 1975.

The intestines were excised, split lengthwise, washed in physiological saline, and the mucosal side scraped with a glass slide. These intestinal scrapings were pooled within treatment groups and cytosol fractions obtained as described for the hepatic tissue. Cytosol fractions were applied to 2.6 X 50 centimeter columns packed with Sephadex G-75 (Pharmacia, Inc., 800 Centennial Avenue, Piscataway, New Jersey 08854). Fractionation was performed at a flow rate of 0.5 ml per minutes and at 4° C using a buffer containing 0.9 percent sodium chloride, 0.02% sodium azide, and 10 millimolars TRIS-hydrogen chloride (pH = 8.6) as the elution fluid. Three-ml fractions were collected; the elutions were aspirated directly into the atomic absorption spectrophotometer.

Flow Cytometry Analysis. After removing the one-ml aliquot of whole blood for determination of zinc and copper concentration, the remaining 11 ml of whole blood was processed for analysis by flow cytometry. The blood was diluted with an equal volume of 1X Hanks Balanced Salt Solution (Gibco Laboratories, Chagrin Falls, Ohio 4402) supplemented with 3.5 g sodium bicarbonate, 5.0 mg ethylenediaminetetra-acetic acid, and 2.86 ml of a 35-percent solution of bovine serum albumin (HBSS). The diluted blood was separated into equal volumes, layered on top of 4 ml of Ficoll-Paque (Pharmacia Fine Chemicals, Division of Pharmacia, Inc., Piscataway, New Jersey 08854) and centrifuged at 400 X g for 20 minutes at 10° C. The cells at the interface were removed with a Pasteur pipette, diluted with 12 ml HBSS and centrifuged for 10 minutes at 200 X g at 10° C. The supernatant was decanted and the cell pellet suspended in one ml HBSS.

Two ml lysing solution (4.15 g sodium chloride, 0.5 g potassium bicarbonate, and 0.185 g sodium ethylenediaminetetra-acetic acid in 500 ml distilled water, pH = 7.3) was added and the tubes set aside for 15 minutes at room temperature. Fetal calf serum (0.8 ml) was layered beneath the diluted cells and the tubes centrifuged for 20 minutes at 200 X g at 10° C. The supernatant was thus aspirated and the cell pellet suspended with HBSS and washed twice as previously described.

A Coulter Counter (Model ZM, Coulter Electronics Limited, England) was used to determine the total number of cells recovered. Each sample was then diluted to 1 x 10^7 cells per ml with HBSS.

Fifty microliters (µl) of the diluted cell suspensions were pipetted into 96 well microtiter plates. Mouse monoclonal antibodies that bind to surface antigens of rat T-, T-helper, and T-suppresser lymphocytes (Pel-Freez Biologicals, Post Office Box 68, Rogers, Arkansas 72756) were added to the appropriate wells. The plates were placed on ice for 15

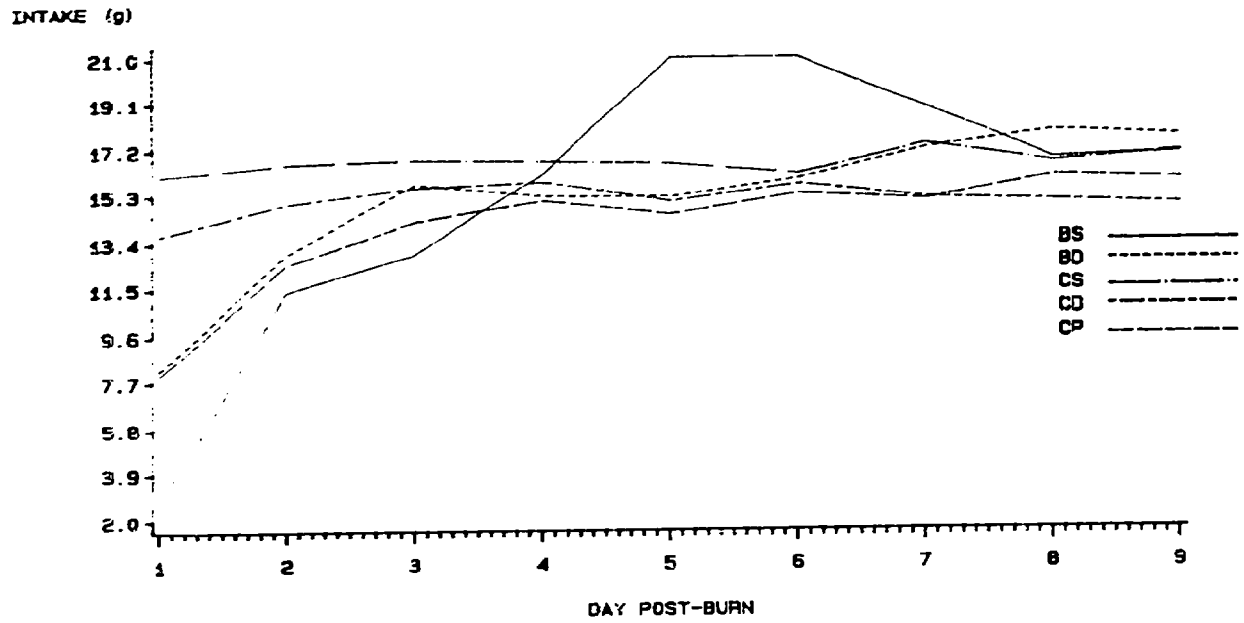


FIGURE 1. Feed intake for the 10 days following burn injury for rats assigned to the following treatment regimens: BS = burned, zinc supplemented, fed ad libitum, BD = burned, not zinc supplemented, fed ad libitum, CS = not burned, zinc supplemented, fed ad libitum, CD = not burned, not zinc supplemented, fed ad libitum, and CP = not burned, zinc supplemented, pair-fed to BD group.

minutes and then centrifuged at 200 X g for five minutes. The supernatant was aspirated and 50 µl of Goat F(ab')2 anti-mouse immunoglobulin G flourescene-labeled antibody (TAGO, Inc., Post Office Box 4463, 887 Mitten Road, Burlingame, California 94011) was added to each well. The plates were placed on ice for 15 minutes and then centrifuged for five minutes. The supernatant was aspirated and 50 µl HBSS was added to each well and centrifuged at 200 X g for five minutes at 10° C. The supernatant was aspirated and the labeled cells were fixed in 100 µl of a 1:2 solution of two-percent paraformaldehyde. The percentages of T-, T-helper, and T-suppressor cells were determined using a flow cytometer (Model FACS 400, Becton-Dickinson Company, 2375 Garcia Avenue, Mountain View, California 94043).

Statistical Analysis. A computer software program (Version 4.10 (1985), Statistical Analysis System, SAS Institute, Inc., Cary, North Carolina 27511) was used to obtain descriptive statistics and perform one-way analysis of variance. When the F statistic was found to be significant ($P < 0.05$), a Duncans' Multiple Range Test (SAS User's Guide (1982)) was used to test for differences among treatment means.

RESULTS

The weight changes of the rats placed in the metabolic cages from the burn day until day of sacrifice are shown in Table 2. The CS group gained a mean of +10 g over the 10-day period as opposed to +6, +1, -2, and +1 g in the CD, CP, BS, and BD treatment groups, respectively.

Food intake of the BS and BD groups decreased on the first and second postburn days (Figure 1); however, by the third postburn day, food intake for both burn groups was comparable to or greater than the CS and CD groups for the remainder of the study period.

The total obligatory losses of zinc by both fecal and urinary routes of excretion showed no significant ($P < 0.05$) difference due to burn injury when the burned groups were compared to their respective nonburn control groups (Table 2). The pair feeding regimen did not cause a significant difference in zinc loss when compared to the zinc sufficient nonburn control group.

The zinc restriction regimen significantly ($P < 0.05$) lowered plasma zinc concentration in both the BD and the CD groups compared to the supplemented groups. Plasma copper concentration was significantly elevated in the BS and BD groups when compared to the three nonburn control groups. Hepatic zinc concentration was significantly increased ($P < 0.05$) in the burn-injured rats that were supplemented with

TABLE 2

BODY WEIGHT AND ZINC EXCRETION DATA (n = 6)

Treatment[a]	Start Body Weight (Grams)	End Body Weight (Grams)	Body Weight Change (Grams)	Fecal Zinc (5 Grams)	Urine Zinc (5 Grams)	Fecal + Urine Zinc (5 Grams)
BS	350 (6)	348 (6)[b]	- 2 (3)[b]	2683 (37)[c]	93 (11)	2683 (37)[c]
BD	349 (8)	349 (8)[b]	+ 1 (2)[b]	908 (75)	22 (2)	930 (76)
CS	352 (6)	362 (4)[d]	+10 (5)[d]	2892 (71)[c]	90 (13)[c]	2982 (61)[c]
CD	353 (12)	358 (10)	+ 6 (3)	896 (96)	33 (4)	929 (94)
CP	351 (9)	352 (9)[b]	+ 1 (3)[b]	2822 (79)[c]	94 (11)[c]	2916 (72)[c]

( ) = ± standard error.

[a]Treatment Groups: BS = burned, zinc supplemented, fed ad libitum.
BD = burned, not zinc supplemented, fed ad libitum.
CS = not burned, zinc supplemented, fed ad libitum.
CD = not burned, not zinc supplemented, fed ad libitum.
CP = not burned, zinc supplemented, pair-fed to BD group.

[b]Significantly different from CS group at $P < 0.05$.

[c]Significantly different from BD and CD groups at $P < 0.05$.

[d]Significantly different from BS, BD, and CP groups at $P < 0.05$.

zinc (Table 3). No significant differences were found between the BD and CD treatment groups.

Fractionations of cytosol preparations from hepatic tissue are shown in Figures 2 through 5 and from intestinal mucosal tissue in Figures 6 through 9. Burn injury caused a sharp increase in zinc bound to a 12,000 MW protein in the hepatic cytosol of rats supplemented with zinc, but not in either the rats maintained on the zinc deficient regimen or the control groups. There was no increase in zinc bound to low MW proteins in intestinal mucosal cytosol in any of the treatment groups; however, there was an increase in zinc bound to a protein that eluted in the void volume of the burned rats that were supplemented with zinc.

The peripheral leukocyte blood cell data are shown in Table 4. Burn injury caused a significant leukocytosis, irrespective of zinc supplementation. Based on the data from flow cytometer analysis (Table 4), no significant differences among the treatment groups with respect to the percentages of T-cells or T-helper cells could be found. There was a significant ($P < 0.05$) increase in T-suppressor cells in the BD group over the other four treatment groups when the data were expressed as either percent of fluorescent positive cells or as total cells per ml of whole blood.

DISCUSSION

Previous studies have shown that a 30-percent TBSA burn caused sequestration of zinc in hepatic tissue (7), even during deficient zinc intake. To create the zinc deficiency in those studies the rats were subjected to a fasting regimen during the postburn injury period, making it difficult to separate the effect of total malnutrition from burn injury. Our results show that rats allowed to maintain nutrient intake and given adequate zinc supplementation sequester zinc in hepatic tissue subsequent to burn injury, while zinc deficient but otherwise well nourished rats had significantly lower levels of hepatic zinc concentration when compared to nonburned ad libitum or pair-fed zinc sufficient control rats.

To further characterize the changes that occur in zinc metabolism during recovery from a burn injury, we analyzed the protein bound zinc in mucosal intestinal and hepatic cytosol using gel column chromatography. There has been extensive research using similar procedures that suggest an important role of metallothionein, a low MW protein, in the homeostatic

TABLE 3

PLASMA AND LIVER ZINC AND COPPER CONCENTRATION (n = 12)

Treatment[a]	Plasma Zinc	Plasma Copper	Liver Zinc	Liver Copper
BS	137 (7)[b]	132 (7)[c]	162 (111)[d]	17 (1)
BD	68 (5)	127 (5)[c]	89 (8)[e]	17 (2)
CS	143 (6)[b]	99 (5)[f]	135 (7)[f]	16 (2)
CD	54 (3)	86 (4)[g]	95 (9)[h]	14 (1)
CP	146 (4)[b]	104 (4)[f]	130 (8)[f]	13 (2)

( ) = ± standard error.

[a]Treatment Groups: BS = burned, zinc supplemented, fed ad libitum.
BD = burned, not zinc supplemented, fed ad libitum.
CS = not burned, zinc supplemented, fed ad libitum.
CD = not burned, not zinc supplemented, fed ad libitum.
CP = not burned, zinc supplemented, pair-fed to BD group.

[b]Significantly different from BD and CD groups at $P < 0.05$.

[c]Significantly different from CS, CD, and CP groups at $P < 0.05$.

[d]Significantly different from BD, CS, CD, and CP groups at $P < 0.05$.

[e]Significantly different from BS, CS, and CP groups at $P<0.05$.

[f]Significantly different from BS, BD, and CD groups at $P < 0.05$.

[g]Significantly different from BS, BD, and CP groups at $P < 0.05$.

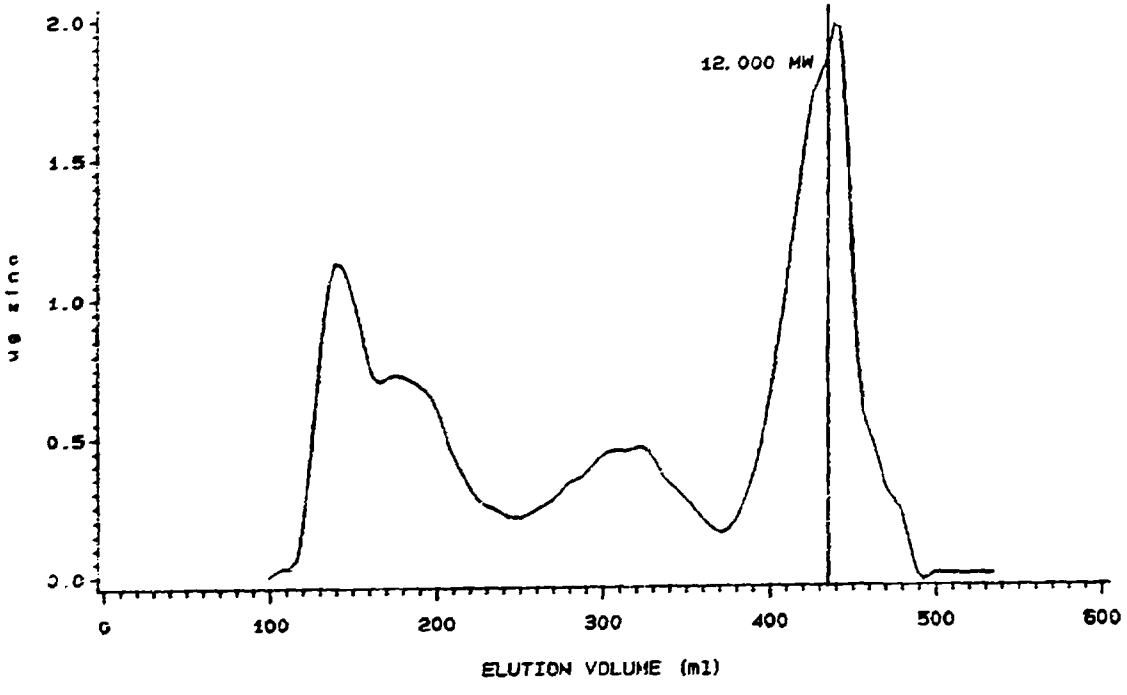


FIGURE 2. Zinc bound to hepatic cell cytosol proteins separated by gel column chromatography (2.6 X 50 centimeter column packed with Sephadex G-75 at a flow rate of 0.5 milliliters per minute at 4° C) for the BS treatment group.

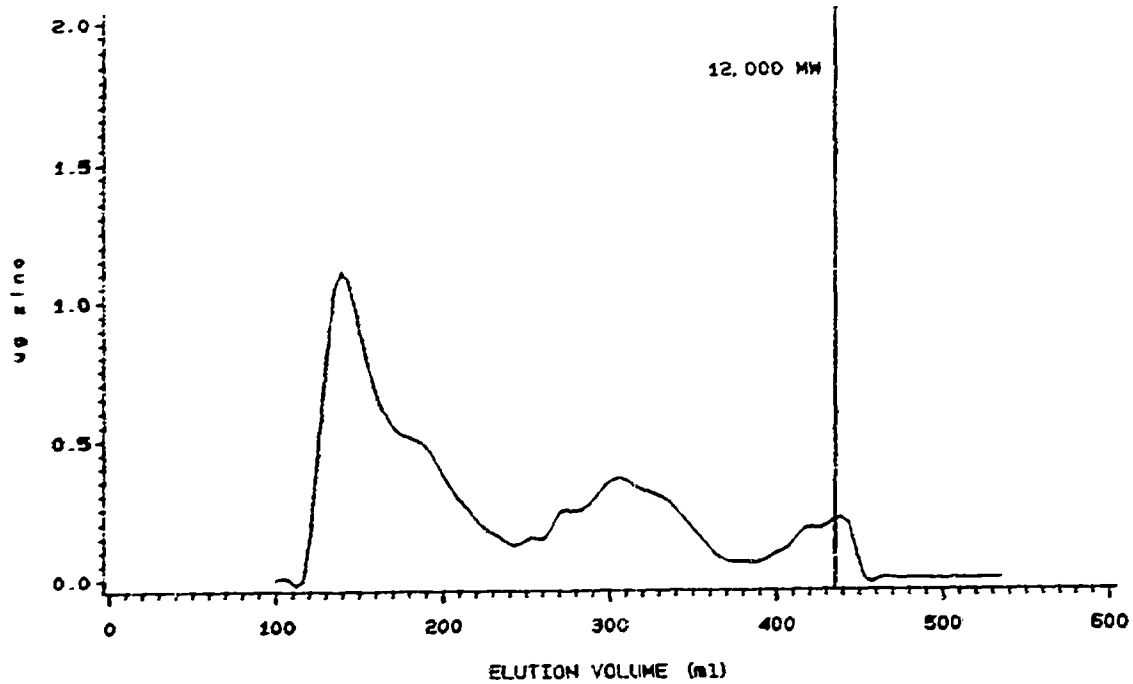


FIGURE 3. Zinc bound to hepatic cell cytosol proteins separated by gel column chromatography (2.6 X 50 centimeter column packed with Sephadex G-75 at a flow rate of 0.5 milliliters per minute at 4° C) for the CS treatment group.

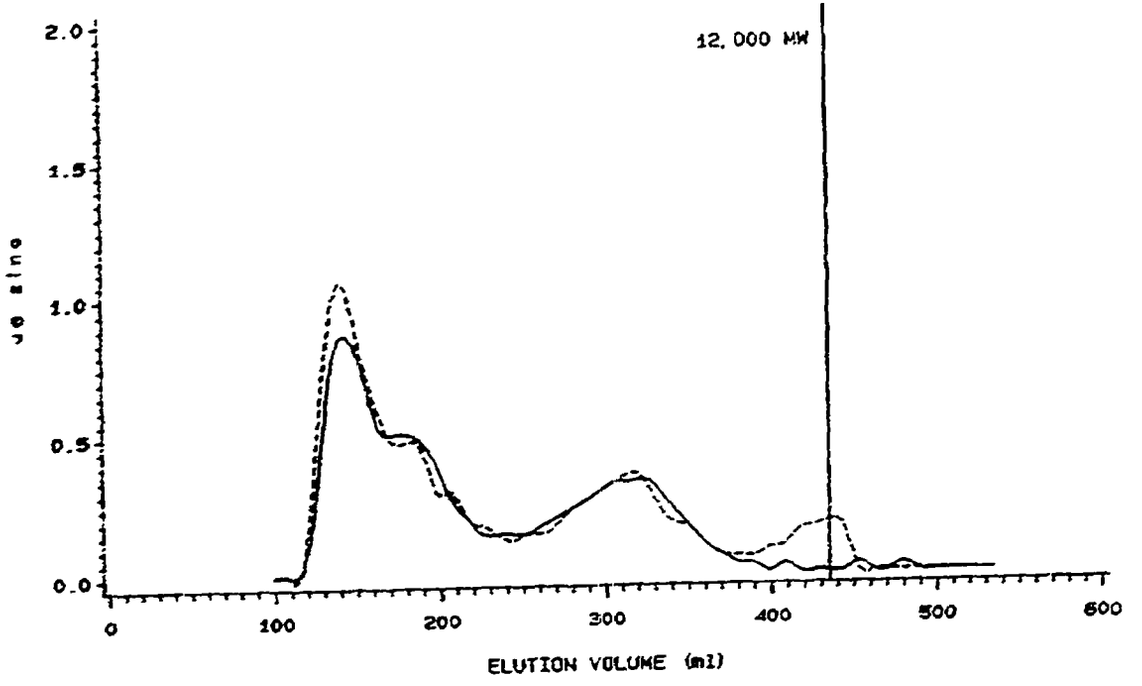


FIGURE 4. Zinc bound to hepatic cell cytosol proteins separated by gel column chromatography (2.6 X 50 centimeter column packed with Sephadex G-75 at a flow rate of 0.5 milliliters per minute at 4° C) for the BD (solid line) and CP (dotted line) treatment groups.

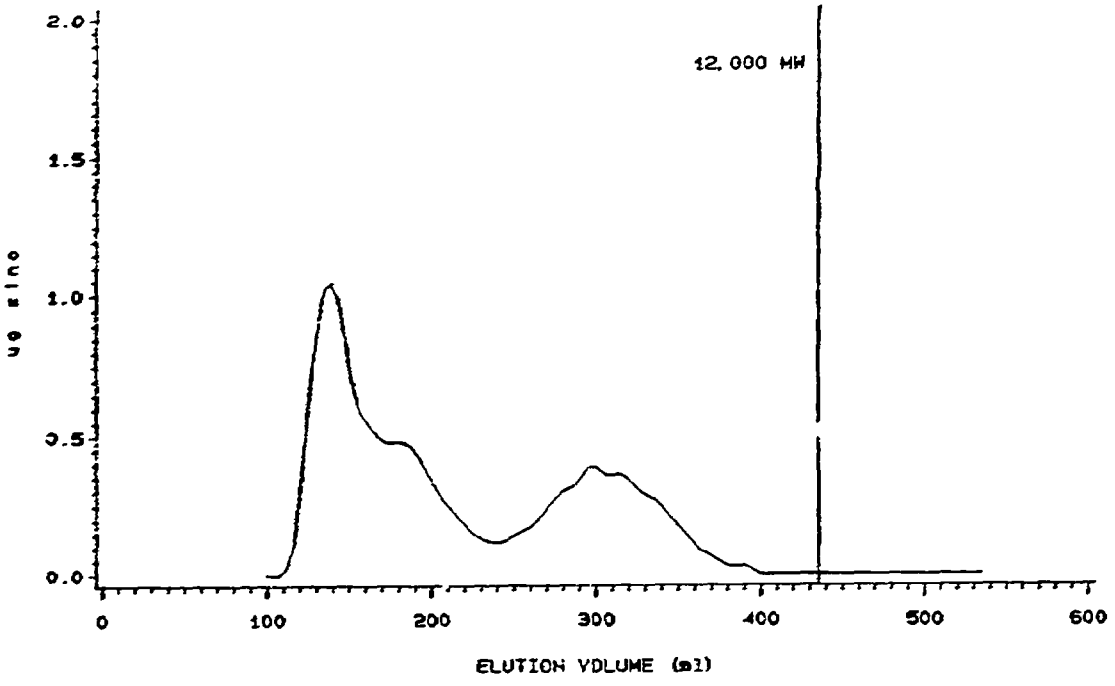


FIGURE 5. Zinc bound to hepatic cell cytosol proteins separated by gel column chromatography (2.6 X 50 centimeter column packed with Sephadex G-75 at a flow rate of 0.5 milliliters per minute at 4° C) for the CD treatment group.

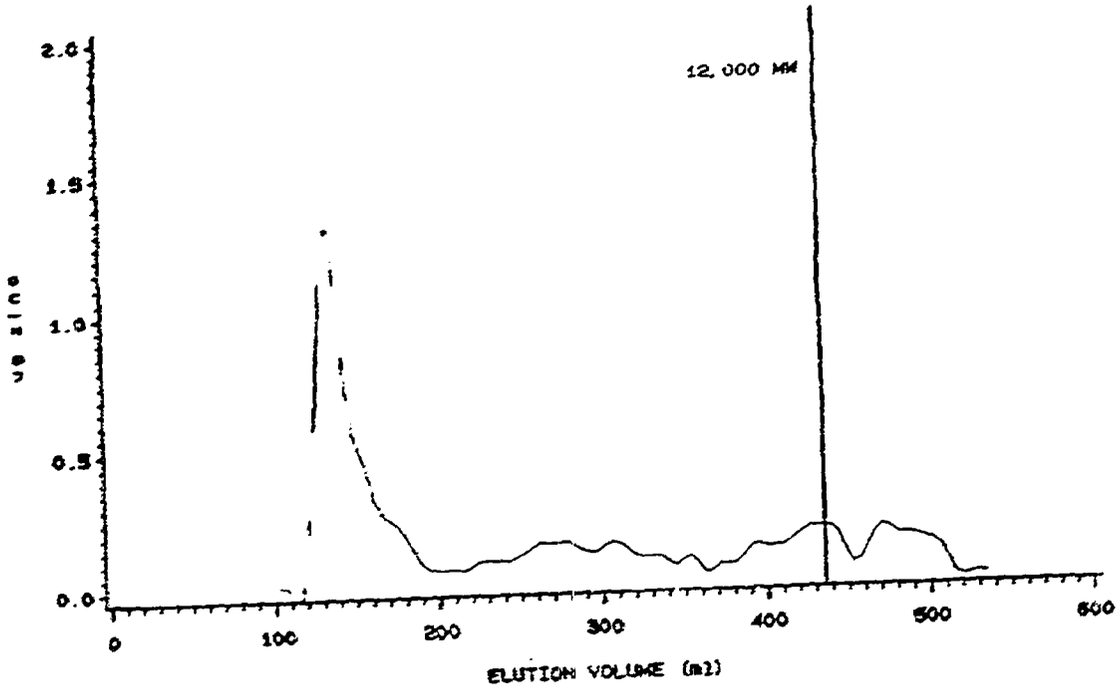


FIGURE 6. Zinc bound to intestinal mucosal cell cytosol proteins separated by gel column chromatography (2.5 X 50 centimeter column packed with Sephadex G-75 at a flow rate of 0.5 milliliters per minute at 4° C) for the BS treatment group.

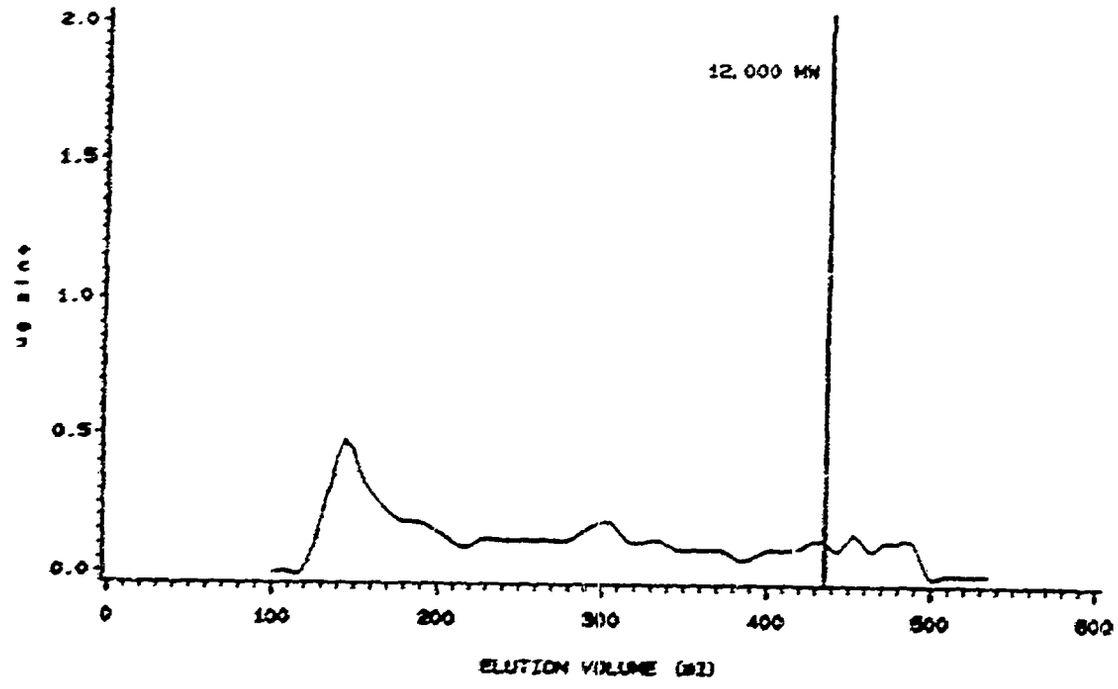


FIGURE 7. Zinc bound to intestinal mucosal cell cytosol proteins separated by gel column chromatography (2.6 X 50 centimeter column packed with Sephadex G-75 at a flow rate of 0.5 milliliters per minute at 4° C) for the CS treatment group.

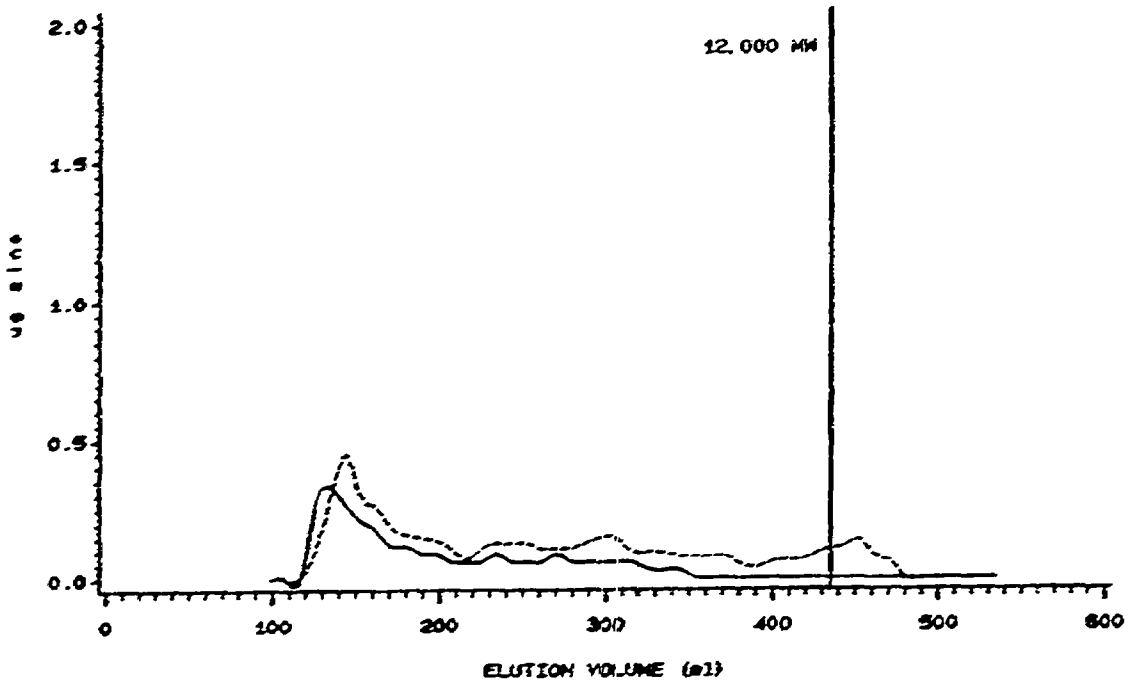


FIGURE 8. Zinc bound to intestinal mucosal cell cytosol proteins separated by gel column chromatography (2.6 X 50 centimeter column packed with Sephadex G-75 at a flow rate of 0.5 milliliters per minute at 4° C) for the BD (solid line) and CP (dotted line) treatment groups.

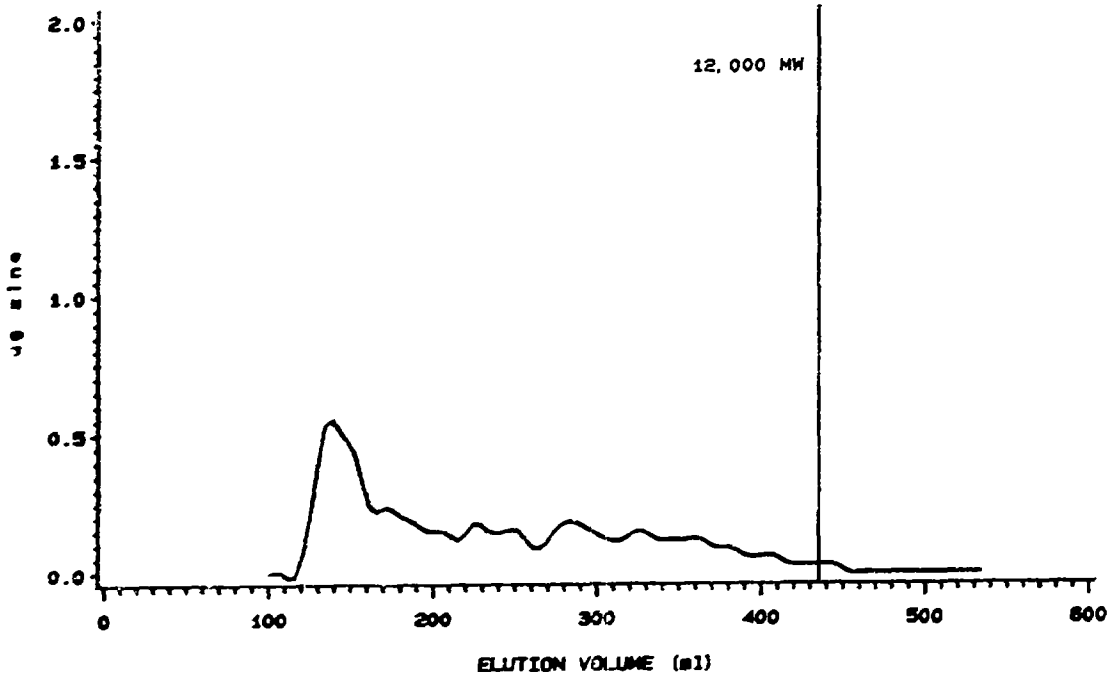


FIGURE 9. Zinc bound to intestinal mucosal cell cytosol proteins separated by gel column chromatography (2.6 X 50 centimeter column packed with Sephadex G-75 at a flow rate of 0.5 milliliters per minute at 4° C) for the CD treatment group.

TABLE 4

PERIPHERAL BLOOD CELL DATA (n = 12)

Treatment[a]	White Blood Cells	PERCENT[b]				ABSOLUTE[b]		
		Lymphocytes	T-Cell	T-Helper	T-Suppressor	T-Cell	T-Helper	T-Suppressor
BS	6100 (472)[b]	68 (2)[c]	66 (2)	44 (2)	22 (2)	2766 (279)	1845 (199)	948 (171)
BD	6035 (385)[d]	71 (3)[c]	66 (2)	40 (1)	32 (1)[e]	2896 (310)	1728 (152)	1430 (177)[e]
CS	4255 (293)	75 (3)	70 (2)	45 (2)	21 (1)	2298 (224)	1423 (157)	696 (88)
CD	3778 (332)	82 (3)[f]	67 (4)	43 (3)	22 (2)	2219 (312)	1424 (213)	723 (111)
CP	4625 (490)	80 (2)[f]	73 (1)	46 (1)	25 (1)	2789 (397)	1689 (223)	913 (129)

( ) = ± standard error.

[a]Treatment Groups: BS = burned, zinc supplemented, fed ad libitum.
BD = burned, not zinc supplemented, fed ad libitum.
CS = not burned, zinc supplemented, fed ad libitum.
CD = not burned, not zinc supplemented, fed ad libitum.
CP = not burned, zinc supplemented, pair-fed to BD group.

[c]Significantly different from CD and CP groups at $P < 0.05$.

[d]Significantly different from CS, CD, and CP groups at $P < 0.05$.

[e]Significantly different from BS, CS, CD, and CP groups at $P < 0.05$.

[f]Significantly different from BS and BD groups at $P < 0.05$.

control of zinc metabolism (16). This protein elutes at the 12,000 MW range and is synthesized in both liver and intestinal tissue (17). Most such research has used high levels of zinc or cadmium intake to induce metallothionein synthesis in experimental animal models. While a few reports have described the effects of bacterial lipopolysaccharides or hypersensitivity on metallothionein induction, relativly little research has been reported on the role of this protein in zinc metabolism during specific disease or after trauma.

Based on the fractionation data from the rats maintained on a sufficient zinc regimen, a 30-percent TBSA burn caused an increase binding of zinc to a protein of approximately 12,000 MW in liver cytosol tissue. This phenomenon was not seen in burned, zinc deficient rats. The burn injury in the zinc deficient rats caused neither an increase in hepatic zinc concentration nor a redistribution in protein bound zinc.

It is important to note that the pair-feeding regimen did not cause an increase in hepatic zinc concentration nor in zinc bound to low MW proteins. Food restriction alone has been shown to induce metallothionein zinc binding (18). This may account for the discrepancy between earlier studies (7) and the present results. It is possible that the combination of burn injury and the fasting state induced metallothionein synthesis and increased zinc binding in hepatic tissue.

It has been suggested that there is a relationship between zinc and nitrogen redistribution during burn injury and/or infection (7,19). Davies and Fell (4) found a correlation between the amounts of zinc and creatinine excretion in burn injured humans. Results from the present study argue against this view. If part of the sequestered zinc was from the burned tissue or from another endogenous tissue source due to the burn injury, then the burned/zinc deficient rats should show an increase in liver zinc concentration when compared to the nonburned/zinc deficient rats.

---

[16] Cousins RJ: Absorption, transport, and hepatic metabolism of copper and zinc: special reference to metallothionein and ceruloplasmin. Physiol Rev 65:238-309, 1985.

[17] Danielson KG, Ohi S, and Huang PC: Immunochemical detection of metallothionein in specific epithelial cells of rat organs. Proc Natl Acad Sci USA 79:2301-2304, 1982.

[18] Bremmer I and Davies NT: The induction of metallothionein in rat liver by zinc injection and restriction of food intake. Biochem J 149:733-738, 1975.

[19] Davies JWL: Body content of water and electrolytes. In Physiological Responses to Burning Injury. New York: Academic Press, 1982, pp 240-244.

It should be pointed out that the methodology used in our studies only measured the amount of zinc bound to proteins eluted at various MW by gel column chromatography. It would be of interest to use a radioactive-labeled amino acid pulse concurrent with radioactive zinc experiments to determine simultaneous changes in protein synthesis and zinc binding in burned/zinc deficient animals.

The metabolic mechanisms causing an increase in zinc bound to low MW protein in hepatic tissue in the burned/zinc sufficient rats failed to induce similar effects in intestinal cytosol. Cousins (16) has proposed a role for intestinal metallothionein in the excretion of zinc that is in excess of metabolic requirements. Intestinal metallothionein is induced in response to zinc loading, binds excess zinc, and accumulates in the mucosal cells. Subsequently, the zinc bound to metallothionein is lost when cells are sloughed into the lumen, thereby increasing fecal endogenous zinc excretion. Consistent with this suggested role of intestinal metallothionein, it could be hypothesized that the lack of increase in metallothionein binding of zinc in the intestinal cytosol of the burned rats would insure unobstructed zinc absorption and decrease obligatory loss of fecal zinc. Our results support this hypothesis in that total endogenous fecal zinc excretion for the 10-day period postburn did not differ significantly between the burned rats and their respective control group.

Along with defining changes in zinc metabolism following burn injury, we also investigated the possibility of an interaction between zinc nutriture, burn injury, and immunocompetence. Based on monoclonal antibody labeling and flow cytometry analysis, zinc restriction during recovery from burn injury caused a significant increase in T-suppressor lymphocytes. This phenomenon was not seen in the burned, zinc sufficient, nonburned zinc deficient, or pair-fed zinc sufficient rats.

Increased suppressor cells and suppressor cell activity have been described in a number of clinical studies of burns and other trauma (20-22). It is difficult to assess possible nutritional effects on circulating lymphocytes because dietary intake and vitamin and mineral supplementation were not controlled in these studies. Recent animal studies support increased suppressor activity due to burn injury (23), but again, nutritional support was not described.

Although impairment of immunological function in zinc-deficient animals has been well documented, there has been a lack of definitive information at the molecular level that zinc is directly involved. Recently, it has been shown that the biological activity of thymulin, a nonapeptide hormone produced by thymic epithelial cells, is dependent on the

presence of zinc in the molecule (13), and this finding may, in part, explain the relationships.

PRESENTATIONS/PUBLICATIONS

None.

---

[20]O'Mahony JB, Palder SB, Wood JJ, et al: Depression of cellular immunity after multiple trauma in the absence of sepsis. J Trauma 24:869-875, 1984.

[21]Ozkan AN and Ninnemann JL: Circulating mediators in thermal injuries: isolation and characterization of a burn injury-induced immunosuppressive serum component. JBCR 6:147-151, 1985.

[22]Ninnemann JL: The immunology of injury: part one. JBCR 6:128, 1985.

[23]Kupper TS and Green DR. Immunoregulation after thermal injury: sequential appearance of I-J+, Ly-1 T suppressor inducer cells and Ly-2 T suppressor effector cells following thermal trauma in mice. J Immmunol 133:3047-3053, 1984.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA309172	2. DATE OF SUMMARY: 86 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 85 10 01	4. KIND OF SUMMARY: D	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DISB'N INSTR'N: CX	9. LEVEL OF SUM A. WORK UNIT:

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	079
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) Derivative Spectroscopy Chemiluminigenic Probing of Granulocyte Redox Function in Healthy Controls and Burn Patients

12. SUBJECT AREAS
06 01 Biochemistry 06 05 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
85 10	CONT	DA	C

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED APPROVED BY [signature]
a. DATE EFFECTIVE
b. CONTRACT/GRANT NUMBER
c. TYPE
d. AMOUNT
e. KIND OF AWARD
f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	0.7	47
87	0.7	120

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: ALLEN, R C
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-7832
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (if available): JADWIN, D F g. NAME OF ASSOCIATE INVESTIGATOR (if available): MASON, A D

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Chemiluminescence; (U) Granulocyte Function; (U) Derivative Spectroscopy; (U) Immunology; (U) Host Resistance;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (Continued) (U) Burns; (U) Volunteers; (U) ILIR; (U) RAII

23. (U) The two major objectives of this study are the development of techniques for quantifying the redox enzyme content and function of granulocyte leukocytes, and the evaluation of the sensitivity and specificity of these techniques for assessing the status of antimicrobial defense in burn patients. Chemiluminigenic probe (CLP) methods for differential assessment of oxidase and peroxidase activities will be further refined and new techniques based on difference and derivative ultraviolet-visible spectroscopy will be developed for quantifying oxidase and peroxidase enzyme content. The strong relationship between granulocyte function and humoral immune status in the burn patient may also require assessment of serum complement.

24. (U) Ultrasensitive and differential assessment of granulocyte oxygenation activity can be achieved by measuring the luminescence resulting from oxygenation of high quantum yield substrates (CLPs). New photon counting instrumentation designed for operation at physiologic temperature (37° C) will allow more rapid assessment of granulocyte function. The recent availability of diode array ultraviolet-visible spectrophotometers allows for rapid spectral analysis over a broad range. Moreover, the digital nature of the data is ideal for microprocessor-assisted derivative spectral analysis of the enzymes responsible for granulocyte redox activity. Photon counting in combination

CONTINUATION OF DD FORM 1498 FOR "DERIVATIVE SPECTROSCOPY CHEMILUMINIGENIC PROBING OF GRANULOCYTE REDOX FUNCTION IN HEALTHY CONTROLS AND BURN PATIENTS"

with derivative spectroscopy will allow analysis of oxidase and peroxidase structure-function relationships. Such information may explain the derangement in granulocyte function responsible for increased susceptibility to infection.

25. (U) 8510 - 8609. The CLP portion of these studies has demonstrated that granulcoyte function is relatively well preserved for up to 20 hours in ethylenediaminetetra-acetic acid-anticoagulated whole blood kept at room temperature (22 to 24 degrees Celsius). These studies also confirm and extend the results of earlier studies that use of different stimulant-chemiluminigenic probe combinations allows differential assessment of oxidase and peroxidase activities. A diode array ultraviolet-visible spectrophotometer has been recently received and is presently being put in working order.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: DERIVATIVE SPECTROSCOPY CHEMILUMINIGENIC PROBING OF GRANULOCYTE REDOX FUNCTION IN HEALTHY CONTROLS AND BURN PATIENTS

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Robert C. Allen, MD, PhD, Lieutenant Colonel, MC
David F. Jadwin, DO, Captain, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: DERIVATIVE SPECTROSCOPY CHEMILUMINIGENIC PROBING OF GRANULOCYTE REDOX FUNCTION IN HEALTHY CONTROLS AND BURN PATIENTS

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

INVESTIGATORS: Robert C. Allen, MD, PhD, Lieutenant Colonel, MC
David F. Jadwin, DO, Captain, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

The primary objective of this study is development of chemiluminescent, ultraviolet-visible absorption and emission spectroscopic techniques for assessing granulocytic leukocyte redox metabolism required for microbicidal oxygenation activity. The granulocyte is the microbicidal effector phagocyte of acute defense. As such, measurement of phagocyte activity allows assessment of microbicidal capacity and also allows measurement of the humoral information systems that control phagocyte function. Once established, these physical-chemical techniques will be clinically tested with regard to sensitivity and specificity for detecting abnormalities in the humoral-phagocyte immune axis following burn injury. None of the spectroscopic equipment arrived during the period of this report. As such, research emphasis has been directed to refinement of the chemiluminigenic probe approach to whole blood testing. The effects of postvenipuncture whole blood age on oxidase activity were tested using phorbol myristate acetate as the chemical stimulus and lucigenin as the chemiluminigenic probe. Myeloperoxidase activity was tested following immune stimulation with complement-opsonified zymosan using luminol as the chemiluminigenic probe. The specificities of these stimulus-chemiluminigenic probe combinations were further tested using superoxide dismutase, an enzymatic scavenger of superoxide produced by the oxidase, and azide, a potent inhibitor of myeloperoxidase. Approximately 100 patient and 25 control blood specimens were measured following routine complete blood count determination. The blood specimens were tested by random selection without regard to clinical status. The half-life studies indicate that granulocyte metabolic functions, assessed as either oxidase or myeloperoxidase

activities, are relatively well preserved over the first 24 hours using ethylenediaminetetra-acetic acid-anticoagulated whole blood specimens incubated between 20 and 24° C. However, after the initial 24-hour period, both oxidase and myeloperoxidase actiivites decayed exponentially. Also, as suggested by previous studies, the myeloperoxidase activity of burn patients is, in general, considerably higher than controls. This appears related to the extent of toxic (azurophilic) granulation observed during cytologic examination of the blood smears. Oxidase activity is below control values. Inhibition studies substantiate the previous conclusion that the immune stimuli-luminol combination provides a measure of myeloperoxidase activity, whereas phorbol myristate acetate-lucigenin measures superoxide and is thus oxidase-dependent.

DERIVATIVE SPECTROSCOPY CHEMILUMINIGENIC PROBING OF GRANULOCYTE REDOX FUNCTION IN HEALTHY CONTROLS AND BURN PATIENTS

INTRODUCTION

Acute immune protection against infecting microbes is provided by the humoral-phagocyte axis of host defense (1-2). The humoral system, composed of the complement pathways as well as antigen-specific immunoglobulins, recognizes the infecting microbe, generates chemotactic signal, and opsonifies (i.e., surface-labels) the microbe. This information directs the migration of the effector granulocyte to the site of infection. Granulocyte contact with immunoglobulin G-labeled and/or C3b-labeled microbes triggers phagocytosis and activation of respiratory burst metabolism (3). The increase in glucose metabolism via the dehydrogenases of the hexose monophosphate shunt as well as nonmitochondrial oxygen (O_2) consumption results from activation of NAD(P)H:O_2 oxidoreductase, commonly referred to as NAD(P)H oxidase (4). This oxidase catalyzes the univalent reduction of O_2 to hydrodioxylic acid ($.O_2H$) with dissociation yielding superoxide ($.O_2^-$) (5-6). As such, the oxidase changes the spin multiplicity of O_2 from triplet to doublet. Further reactions involving doublet multiplicity

---

[1]Alexander JW, McClellan MA, Ogle CK, et al: Consumptive opsoninopathy: possible pathogenesis in lethal and opportunistic infections. Ann Surg 184:672-678, 1976.

[2]Allen RC and Pruitt BA Jr: Humoral-phagocyte axis of immune defense in burn patients: chemiluminigenic probing. Arch Surg 117:133-140, 1982.

[3]Sbarra AJ and Karnovsky ML: The biochemical basis of phagocytosis. I. Metabolic changes during the ingestion of particles by polymorphonuclear leukocytes. J Biol Chem 234:1355-1362, 1959.

[4]Klebanoff SJ and Clark RA: The Neutrophil: Function and Clinical Disorders. Amsterdam: North-Holland Publishing Company, 1978, pp 1-152.

[5]Allen RC, Stjernholm RL, and Steele RH: Evidence for the generation of an electronic excitation state(s) in human polymorphonuclear leukocytes and its participation in bactericidal activity. Biochem Biophys Res Commun 47:679-684, 1972.

[6]Babior BM, Kipnes RS, and Curnutte JT: Biological defense mechanisms: the production by leukocytes of superoxide, a potential bactericidal agent. J Clin Invest 52:741-744, 1973.

radicals can yield potent oxidizing agents and singlet multiciplicity hydrogen peroxide (H_2O_2) (7-8).

Myeloperoxidase (MPO) is a halide:H_2O_2 oxidoreductase comprising more than five percent of the dry weight of the granulocyte (9). It is an acid-optimum constituent of the azurophil (primary) granule, and in the environment of the phagolysosome, serves as a potent, broad-spectrum microbe-killing system (10-11).

In previous reports, a chemiluminigenic probe (CLP) approach was described which allowed sensitive and differential measurement of oxidase and MPO activities (12-13). The approach is based on the measurement of the light, i.e., chemiluminescence, generated as an energy product of dioxygenation reactions. Introduction of high quantum yield substrates with different chemical reactivities allows very high measurement sensitivity and a degree of reactive specificity (14). Still greater specificity in differentiating oxidase from MPO is achieved by selective stimulation of granulocytes. Immune-opsonified particles are recognized via granulocyte receptor mechanisms; phagocytosis, azurophilic degranulation, and true phagolysosome formation follows. As

---

[7]Allen RC: Phagocytic leukocyte oxygenation activitis and chemiluminescence: a kinetic approach to analysis. Meth Enzymol 133:449-493, 1986.

[8]Khan AU: Singlet molecular oxygen from superoxide anion and sensitized fluorescence of organic molecules. Science 168:476, 1970.

[9]Schultz J and Kaminker K: Myeloperoxidase of the leucocyte of normal human blood. I. Content and localization. Arch Biochem 96:465-467, 1962.

[10]Klebanoff SJ: Myeloperoxidase-halide-hydrogen peroxide antibacterial system. J Bact 95:2131-2138, 1968.

[11]Klebanoff SJ: Myeloperoxidase: contribution to the microbicidal activity of intact leukocytes. Science 169:1095-1097, 1970.

[12]Allen RC and Loose LD: Phagocytic activation of a luminol-dependent chemiluminescence in rabbit alveolar and peritoneal marcrophages. Biochem Biophys Res Commun 69:245-252, 1976.

[13]Allen RC: Lucigenin chemiluminescence: a new approach to the study of polymorphonuclear leukocyte redox activity. In: Bioluminescence and Chemiluminescence, Basic Chemistry and Analytical Applications. DeLuca MA and McElroy WD (eds). New York: Academic Press, 1981, pp 63-73.

[14]Allen RC: Biochemiexcitation: chemiluminescence and the study of biological oxygenation reactions. In: Chemical and Biological Generation of Excited States. Adam W and Cilento G (eds). New York: Academic Press, Inc., 1982, pp 309-344.

such, MPO is an active participant in dioxygenation activity. On the other hand, chemical stimuli such as phorbol myristate acetate (PMA) activate the oxidase, but there is no phagocytosis (15-16). Thus, the role of MPO is questionable.

Research during this period included additional studies of CLP selectivity in differentiating oxidase from MPO activity. Luminol and lucigenin were employed as CLPs and complement-opsonified zymosan (OZ) and PMA were employed as stimuli, respectively. Superoxide dismutase was employed as an enzymatic scavenger of $\cdot O_2^-$, a product of oxidase activity. Azide was used as an inhibitor of the MPO system. In addition, the effect of postvenipuncture age on granulocyte oxidase and MPO function was tested.

MATERIALS AND METHODS

Patient Data. Whole blood specimens submitted for complete blood counts were randomly selected for testing. Control blood specimens were drawn from laboratory staff volunteers. Patient and control blood specimenms were anticoagulated with potassium ethylenediaminetetraacetate (K_3EDTA) and kept at room temperature (20 to 24° C) until tested. Approximately 125 patient specimens and 25 control specimens were tested.

Chemiluminigenic Probing.

Preparation of Phosphate-Buffered Saline with Glucose and Albumin. Phosphate-buffered saline with D-glucose and albumin was prepared by dissolving 8.0 grams (g) NaCl, 0.2 g KCl, 0.62 g KH_2PO_4, and 1.14 g Na_2HPO_4 in 800 milliliters (ml) water. After the salts were in solution, 10 ml of 10% (weight of solute per volume of solution (w/v)) D-glucose and 20 ml five-percent (w/v) albumin were added; the pH was adjusted to 7.3 and the volume was adjusted to one liter. The medium was then filtered to sterility and kept refrigerated (4° C) until used.

Preparation of Complete Veronal Buffer. Complete veronal buffer (CVB) was prepared by dissolving 7.6 g NaCl, 0.33 g KCl, and 1.0 g sodium 5,5-diethylbarbiturate (versonal) to 800 ml water. With constant mixing, 5.6 ml 1.0 N HCl, 5 ml 0.1 M MgCl2, 10 ml 10-percent (w/v) D-glucose, 20 ml 5% (w/v) albumin, and finally 15 ml 0.03 M CaCl2 were added. The medium was adjusted to a pH of 7.3, and the volume was adjusted to one

---

[15]White JG and Estensen RD: Selective labilization of specific granules in polymorphonuclear leukocytes by phorbol myristate acetate. Am J Pathol 75:45-60, 1974.

[16]De Chatelet LR, Shirley PS, and Johnston RB Jr: Effect of phorbol myristate acetate on the oxidative metabolism of human polymorphonuclear leukocytes. Blood 47:545-554, 1976.

liter. The CLPs luminol or lucigenin were added (see preparation of CLP) to a final concentration of 25 and 50 micrometers, respectively. The CLP-containing media were then filtered to sterility, aliquoted to vials, and kept frozen at -70° C until used. This medium is similar to the veronal buffer routinely employed for studies of the complement system (17).

Preparation of Chemiluminigenic Probes. Two different CLPs were employed in these experiments. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), a cyclic hydrazide with a reported quantum yield of 0.01, was prepared as a 20-millimolar (mM) stock concentration in dimethyl sulfoxide. The stock was kept refrigerated in the dark until diluted with water or buffer for testing. The concentration of aqueous luminol is assayed spectrophotometrically based on a mM extinction coefficient of 7.63 at 347 nanometers (18).

Lucigenin (10,10'-dimethyl-9,9'-biacridinium dinitrate, dibenzanthracene) is a water-soluble acridinium salt with a quantum yield comparable to that of luminol. A five-mM solution is prepared in water and kept refrigerated in the dark. The concentration of the working solution is assayed spectrophotometrically based on mM extinctions of 37.3 and 9.65 at 369 and 430 nanometers, respectively (19). Lucigenin has chemical characteristics in common with the viologens, and as such, caution should be exercised when preparing and using this CLP.

Preparation of Opsonified Zymosan. Zymosan A, a preparation of Saccharomyces cerevisiae cell wall, is suspended in normal (0.85% w/v) saline to a concentration of 250 milligrams per deciliter, and heated in a boiling water bath for 20 minutes. After cooling to 22°, the suspension is centrifuged at a relative centrifugal force of 300 for 10 minutes, and the supernatant is discarded. Zymosan is then opsonified by resuspending the pellet in 200 ml fresh-frozen (-70°) pooled sera. Following gentle rotation at 22° for 20 minutes, the suspension is again centrifuge as described above. The supernatant is discarded, an additional 200 ml of sera is added to the pellet, and the incubation and centrifugation

---

[17]Mayer MM: Complement and completion fixation. In: Experimental Immunochemistry, 2nd Edition. (Kabat EA (ed). Springfield: Charles C. Thomas Publisher, 1961, pp 132-162.

[18]Lee J and Seliger HH: Quantum yields of the luminol chemiluminescence reaction in aqueous and aprotic solvents. Photochem Photobiol 15:227-237, 1972.

[19]Totter JR: Light production in alkaline mixture of reducing agents and dimethyleiacrieylium nitrate. Photochem Photobiol 22:203-211, 1975.

steps are repeated. The pellet of opsonified zymosan (OZ) is then washed with 500 ml of normal saline, centrifuged as described above, and the supernatant discarded. This saline wash is repeated twice in order to remove residual protease activity. The washed OZ is then adjusted to the original concentration, aliquoted to storage tubes, and frozen at -70° C until used. This OZ suspension contains 600 ±200 zymosan particles per microliter (µl). Stimulation was initiated by addition of 100 µl of OZ suspension.

Preparation of Phorbol Myristate Acetate. PMA, a cocarcinogen extracted from croton oil, is known to cause specific degranulation of polymorphonuclear leukocytes and activation of redox metabolism. A five-mM stock solution of PMA is prepared in spectral grade dimethyl sulfoxide. This stock solution is further diluted with water to attain the desired concentration of PMA used for stimulation.

Preparation of Whole Blood. Whole blood is collected into evacuated tubes containing K_3EDTA as anticoagulant. Within 30 minutes of testing, a small portion of the well mixed blood (50 µl) was mixed with phosphate-buffered saline with D-glucose and albumin (4.95 ml) to achieve a 1:100 dilution. An aliquot of the diluted specimen (50 µl) was then added to the CVB containing CLP (1.85 ml), and the chemiluminescence response was measured following addition of stimulus at time zero. A complete blood count with differential leukocyte count was performed on all specimens tested. Data with regard to type and number of leukocytes present is required for calculating specific chemiluminescent activities.

Use of EDTA as anticoagulant ensures against complement activation and associated phagocyte stimulation. Adding the diluted bloood specimen to the CVB just prior to testing reversed the effect of EDTA. The CVB contains sufficient CA_2+ and Mg_2+ to nullify the effect of any remaining EDTA present in the relatively small aliquot of diluted whole blood added. In the experiments presented, whole blood was tested at a final dilution of 1:4000.

Photon Counting. Even relatively weak sources of luminescence can be readily measured using the photon counting capacity of standard liquid scintillation counters. Scintillation counters are actually two photon counters operated in coincidence. The coincidence circuitry is designed to measure the shower or pulse of photons resulting from the near stimultaneous relaxation of the multitude of fluorescent molecules excited by a single ionizing radiation event, e.g., beta emission, and to filter out background chemiluminescence. However, in the out-of-coincidehce mode, the instrument is a photon counter capable of measuring single photon events, i.e., chemiluminescence. The newer model scintillation counters are

equipped with high sensitivity, bialkali spectral response photomultiplier tubes. A LS200 Series (Beckman Instruments) scintillation counter equipped with bialkali spectral resopnse photomultiplier tubes operated in the out-of-coincidence mode at the tritium channel settings was employed in all ambient (23-25°).

Calibration. The luminescence velocity measurements, in relative counts per minute, were converted to blue (luminol equivalent) photons per minute by multiplying the relative counts per minute by a photon conversion factor. This conversion factor was established by calibrating the counter with an established blue photon-emitting standard prepared by Seliger. The value of this count-to-photon conversion factor typically is in the range of 10 to 20.

RESULTS

Superoxide Dismutase Inhibition. At the neutral pH of the extracellular space, anion-anion repulsion limits the direct disproportionation of $.O_2^-$. The presence of accumulated $.O_2^-$ can be measured as superoxide dismutase (SOD)-inhibitable cytochrome-reducing activity (6) or SOD-inhibitable lucigenin-dependent chemiluminescence (7,14). The effect of 5, 25, and 125 units of SOD on the luminescence response from 0.5 µl of OZ-stimulated whole blood using 25 µM luminol as CLP is shown in Figure 1. The patient specimen values are depicted by circles and the control values by stars. Figure 2 presents the results of a similar experiment using PMA as stimulus and lucigenin as CLP. The unit concentrations and symbols are the same as for Figure 1 except that the ordinate is expressed as the natural log value in Figure 2. SOD-dependent inhibition of chemiluminescence was observed using the PMA-lucigenin combination, but there was little effect of SOD on the O_2^--luminol response. This is in agreement with the reported effect of SOD using isolated granulocytes (7).

Azide Inhibition. Azide inhibits MPO at very low concentrations, but does not interfere with the oxidase activity responsible for respiratory burst metabolism and $.O_2^-$ generation. In interpreting the results, it should be kept in mind that azide can inhibit other metalloenzymes, especially at relatively high concentrations. Figure 3 depicts the inhibitory effect of 0.2, 1, and 5 µM azide on the luminescence response from 0.5 µl equivalent specimens of whole blood stimulated with OZ using 25 µM luminol as CLP. In Figure 4, the conditions are the same except that PMA was employed as stimulus and 50 µM lucigenin served as the CLP. In both figures, patient specimen data are depicted as circles and control data as stars. Note that the absissa is the natural log of the azide concentration. Azide exerts a dramatic inhibitory effecton the OZ-luminol system, but has practically

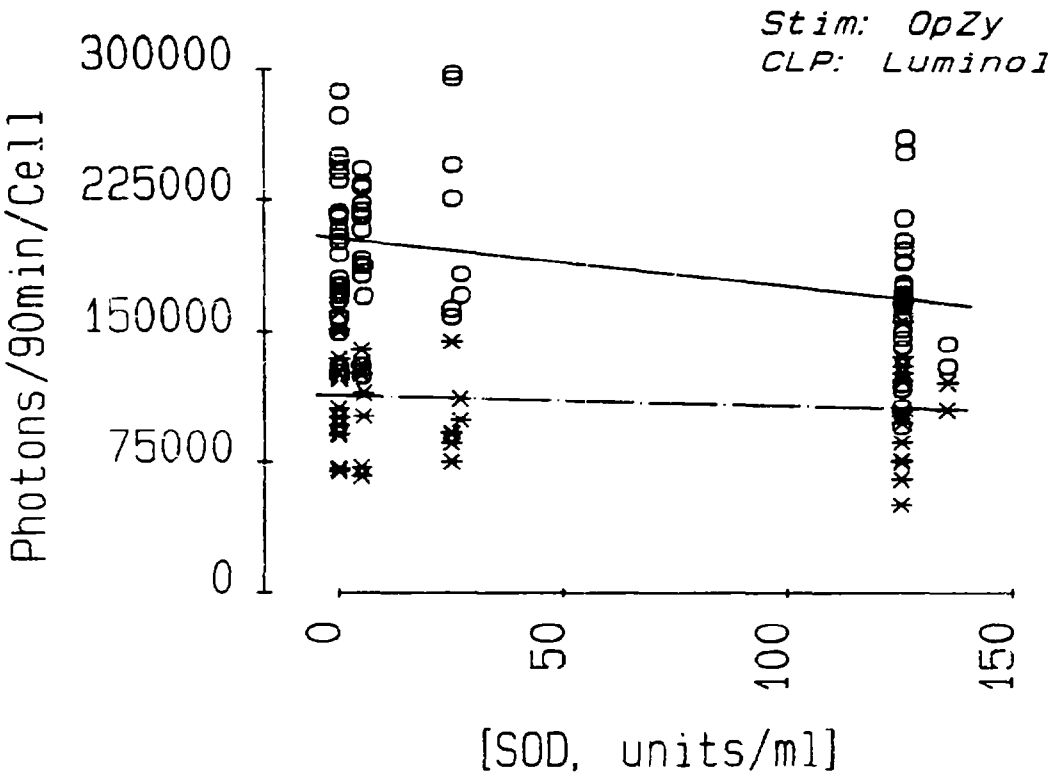


FIGURE 1. Effect of superoxide dismutase (SOD) on the luminol-dependent luminescence responses of opsonified zymosan-stimulated phagocytosis in 0.5 µl equivalent whole blood specimens from patients (open circle) and controls (asterisk).

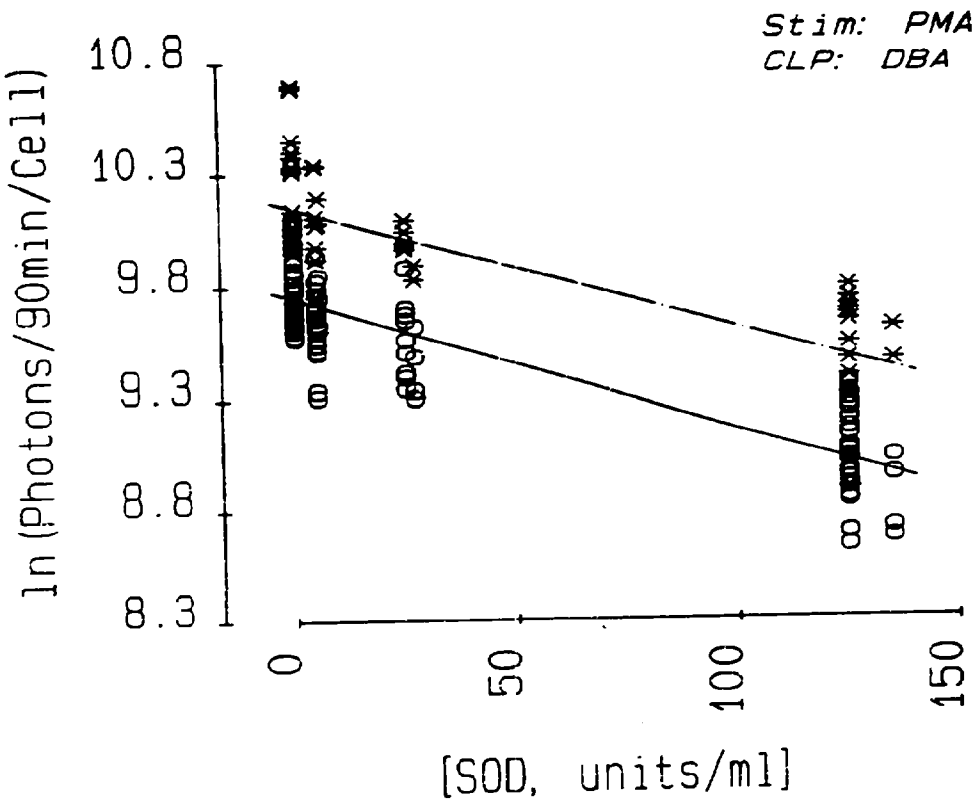


FIGURE 2. Effect of superoxide dismutase (SOD) on the lucigenin-dependent luminescence responses of phorbol myristate acetate-stimulated phagocytes in 0.5 µl equivalent specimens of whole blood. Note that ordinate values are presented as the natural log of luminescent activity.

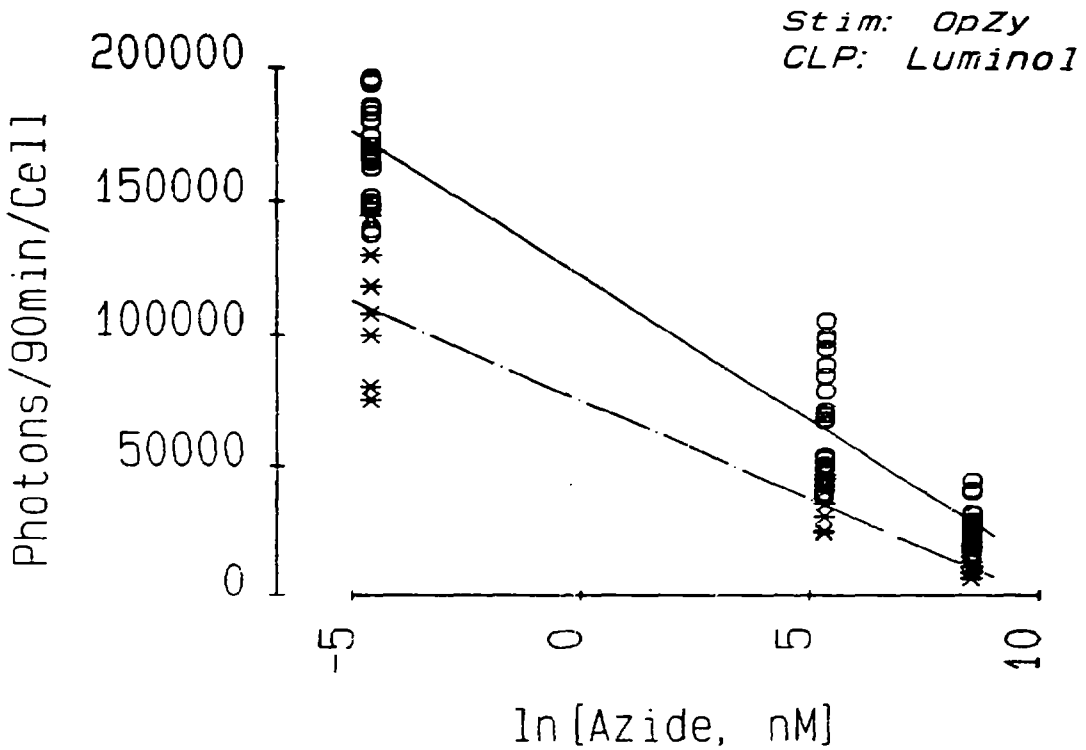


FIGURE 3. Effect of azide on the luminol-dependent luminescence responses of opsonified zymosan-stimulated phagocytes in 0.5 µl equivalent specimens of whole blood. Note that the abscissa is the natural log of the azide concentration.

no effect on the PMA-lucigenin system. These data are consistent with the proposal that the OZ-luminol system measures mostly, but not exclusively, MPO-dependent activity and supports the previously described inhibitory effects of azide on isolated granulocytes (7).

In vitro Lifetime of Granulocytes in Whole Blood. Granulocytes are reported to have a short in vivo lifetime in the circulation (4). The in vitro lifetime of isolated, purified granulocytes is also relatively short (20). Therefore, postvenipuncture age might seriously compromise granulocyte functional measurements from whole blood. The effect of in vitro age on the stimulated luminescence response of unseparated, EDTA-anticoagulated whole blood specimens was tested in order to define the postvenipuncture time limits for testing.

Figures 5 and 6 present the effects of postvenipuncture age on the luminescence responses obtained from 0.5 1 whole blood specimens using OZ-luminol and PMA-lucigenin, respectively. Approximately 100 patients and 25 control blood specimens were tested in duplicate. Note that in both figures, the ordinate values are given in natural log units. During incubation, the whole blood specimens were incubated undiluted at temperatures ranging from 20 to 24° C. Using either stimulus-CLP combination, the responsiveness of whole blood granulocytes is well maintained during the initial 24 hours postvenipuncture. However, the decline in function is exponential for both control and patient specimens, regardless of the stimulus-CLP combination, following the first 24-hour period.

DISCUSSION

The results of these studies support several conclusions drawn from previous investigations using isolated granulocytes and also represent several new conclusions. First, luminol and lucigenin measure MPO and oxidase-dependent activities respectively (7). Second, azide and SOD can be used to inhibit the MPO and $\cdot O_2^-$ dependent luminescent activities of whole blood specimens respectively. Third, granulocyte function, measured using either stimulus-CLP combination, is relatively well maintained in undiluted, EDTA-anticoagulated whole blood during the initial 24-hour postvenipuncture interval, and fourth, disintegration of granulocyte function is an

---

[20]Allen RC and Lint TF: Correlation of chemiluminescence to microbicidal metabolic response from polymorphonuclear leukocytes: a study of in vitro aging. In: Analytical Applications of Bioluminescence and Chemiluminescence. Schram E and Stanley P (eds). Westlake Villege, California: State Printing and Publishing, 1979, pp 589-600.

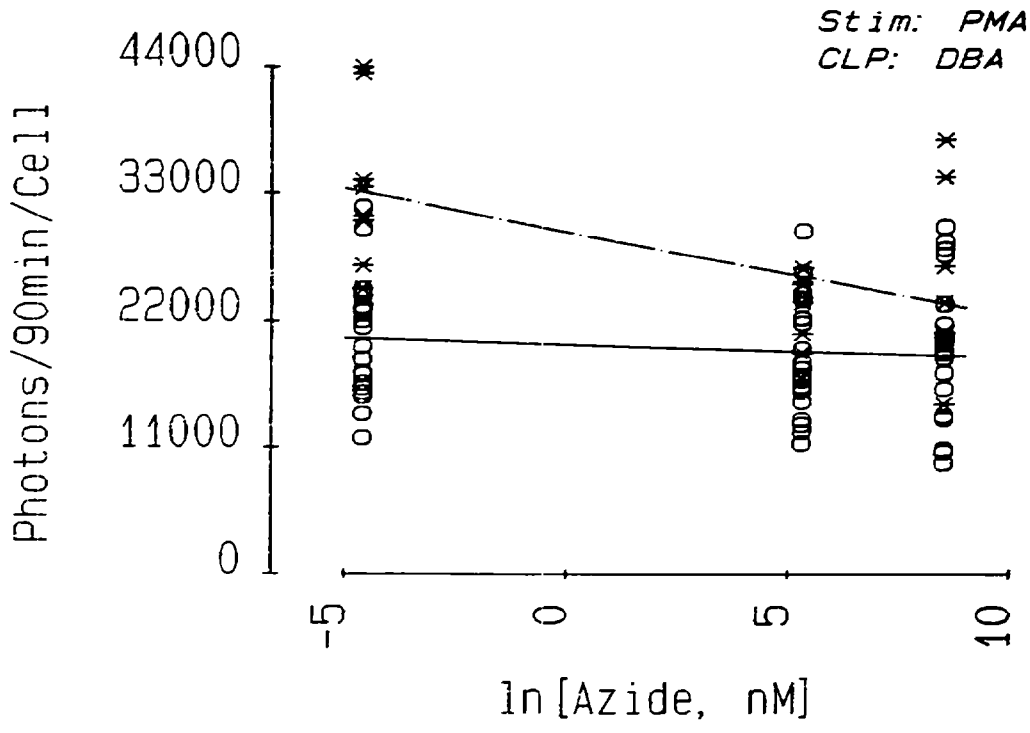


FIGURE 4. Effect of azide on the lucigenin-dependent luminescence responses of phorbol myristate acetate-stimulated phagocytes in 0.5 µl equivalent specimens of whole blood. The abscissa is the natural log of the azide concentration.

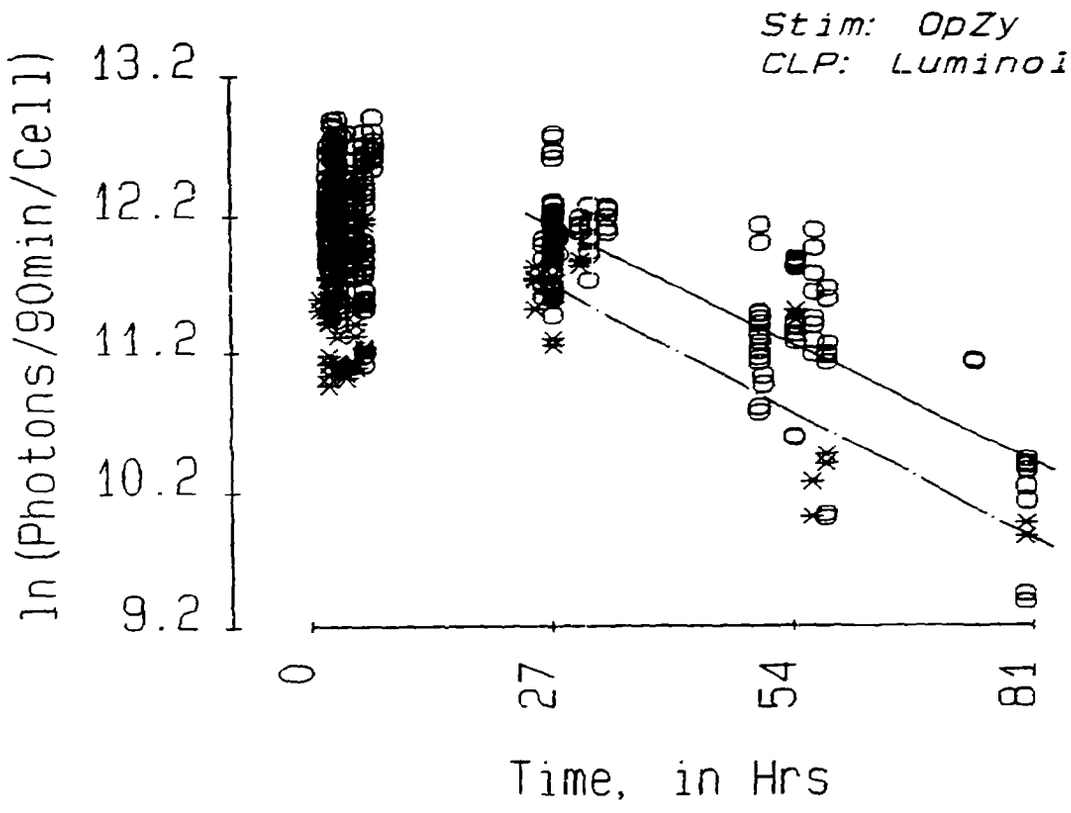


FIGURE 5. Effect of postvenipuncture age on the luminol-dependent luminescence responses of opsonified zymosan-stimulated phagocytes in 0.5 µl equivalent specimens of whole blood from patients (open circle) and controls (asterisk). Note that the ordinate is expressed as the natural log of luminescence activity.

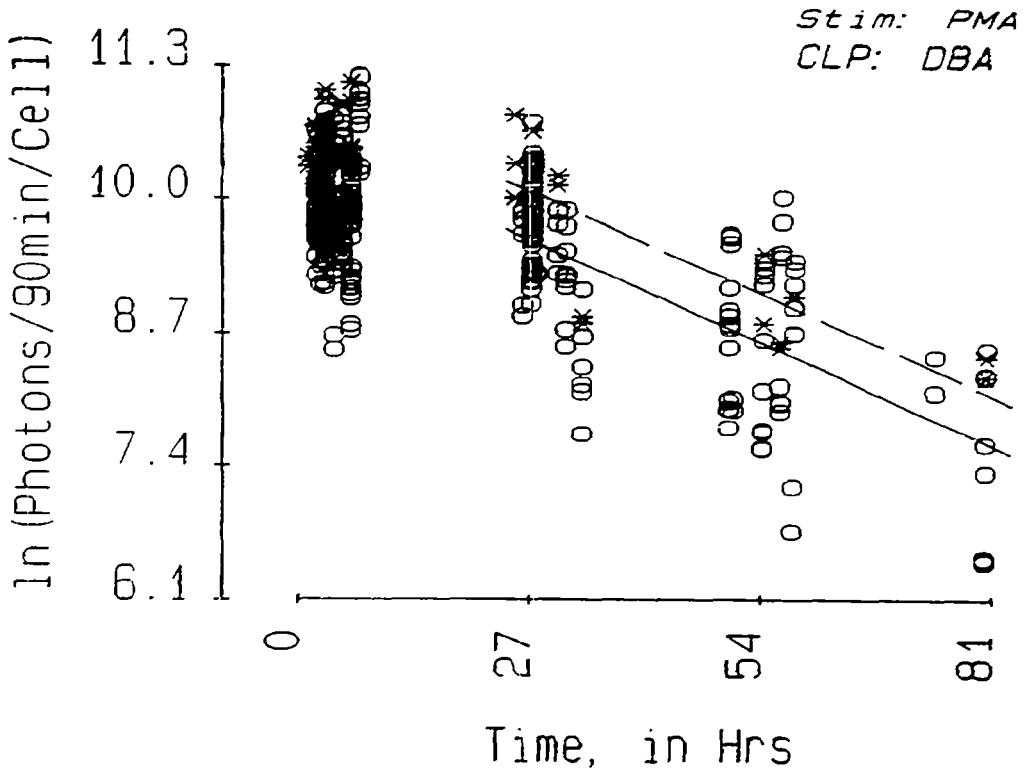


FIGURE 6. Effect of postvenipuncture age on the lucigenin-dependent luminescence responses of phorbol myristate acetate-stimulated phagocytes in 0.5 µl equivalent specimens of whole blood from patients (open circle) and controls (asterisk). Note that the ordinate is expressed as the natural log of luminescence activity.

exponential function of postvenipuncture age following the initial 24-hour plateau interval.

As previously described, granulocytes from burn patients have a high MPO activity relative to controls and this activity is qualitatively related to the degree of toxic granulation observed cytologically. However, oxidase activity in burn patient granulocytes is relatively low in comparison to control granulocytes.

In conclusion, the techniques for chemiluminescence probing of granulocyte oxidase and peroxidase function are ultrasensitive, nondestructive, require relatively little time for analysis, and are relatively stable with respect to postvenipuncture age of specimen. Diminished oxidase function, especially in combination with low MPO activity, may signal an increased clinical susceptibility to infection. If so, the luminescence appraoch described may be of value in supporting clinical evaluation of burn patients and possibly other immunologically compromised patients.

PUBLICATIONS/PRESENTATIONS

Allen RC: Phagocytic leukocyte oxygenation activities and chemiluminescence: a kinetic approach to analysis. Meth Enzymol 133:449-493, 1986

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA311491	86 10 01	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	081
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE *(Precede with Security Classification Code)*
(U) Cultured Keratinocytes as Epithelial Grafts for Burned Soldiers

12. SUBJECT AREAS
06 05 Clinical Medicine 06 13 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
86 10	CONT	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
a. DATE EFFECTIVE APPROVED BY Ronald L. Trunkey
b. CONTRACT/GRANT NUMBER
c. TYPE d. AMOUNT
e. KIND OF AWARD f. CUM/TOTAL

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
86	0.0	0
87	1.5	70

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)* Fort Sam Houston San Antonio, Texas 78234-6200	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR MC MANUS, A T
d. TELEPHONE NUMBER *(Include area code)* 512-221-2720	d. TELEPHONE NUMBER *(Include area code)* 512-221-3411
21. GENERAL USE FINA MILITARY/CIVILIAN APPLICATION M	e. NAME OF ASSOCIATE INVESTIGATOR *(If available)* g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Healing; (U) Keratinocytes; (U) Cell Culture; (U) Skin; (U) Skin Graft; (U) Frozen Skin; (U) Volunteers;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

22. (Continued) (U) ILIR; (U) RAII

23. (U) To evaluate cultured keratinocytes as grafts for epithelial closure of burn wounds. To identify technical and immunological requirements to establish frozen banks of histocompatible keratinocytes for wound coverage.

24. (U) The possible utility of cultured keratinocytes will be established initially with cultured autologous keratinocytes. Keratinocytes will be cultured from biopsies taken early after admission of patients with large burns and limited unburned donor sites for standard partial thickness autografts. If such grafts are deemed cleanly useful, efforts will expand into investigations of allogeneic skin cultures.

25. (U) 8510 - 8609. This is a new project. Orders for equipment and supplies required to begin the study have been placed.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA302498	2. DATE OF SUMMARY: 86 10 01	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 85 10 01	4. KIND OF SUMMARY: K	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING	8. DISP'N INSTR'N: CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	089
b. CONTRIBUTING				
c. CONTRIBUTING	NONE			

11. TITLE (Precede with Security Classification Code) (U) Inequality of VA/Q Ratios Following Smoke Inhalation Injury and the Effect of Angiotensin Analogues

12. SUBJECT AREAS
06 05 Clinical Medicine 06 16 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
83 08	8609	DA	C

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED APPROVED BY David E. Beatty Jr.

a. DATE EFFECTIVE	b. CONTRACT/GRANT NUMBER	c. TYPE	d. AMOUNT	e. KIND OF AWARD	f. CUM/TOTAL

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
86	2.0	67
87	0.0	0

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	e. NAME OF PRINCIPAL INVESTIGATOR: MASON, A D Jr
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-7832
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (If available): SHIMAZU, T g. NAME OF ASSOCIATE INVESTIGATOR (If available): IKEUCHI, H

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Inhalation Injury; (U) Cardiac Output; (U) Indicator Dilution; (U) Ventilation-Perfusion Ratio; (U) Cobra Venom;

23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS (Precede text of each with Security Classification Code)

22. (U) Lab Animals: (U) Sheep; (U) ILIR; (U) RAII

23. (U) To evaluate the effect of smoke inhalation on pulmonary ventilation and perfusion. To study the effects of positive end-expiratory pressure and oxygen on pulmonary ventilation-perfusion ratio. To study the role of the complement system in the respiratory insufficiency following smoke inhalation.

24. (U) The ventilation-perfusion ratio will be measured utilizing the six-inert gas technique. These pulmonary variables will be correlated with standard cardiopulmonary variables before and after the introduction of inhalation injury and subsequent treatment with positive end-expiratory pressure and/or oxygen. Lung lymphatic collection will be used to permit assessment of the pathophysiologic mechanisms of pulmonary edema formation and the contribution of chemical mediators, especially platelet activating factors, to such edema formation after smoke inhalation. Animals decomplemented by cobra venom factors will be studied to assess the role of the complement system in the process of inflammatory responses following smoke inhalation.

25. (U) 8510 - 8609. Ventilation-perfusion alterations following smoke inhalation injury have been established. Techniques for lung lymph collections and blood platelet activating factor preparation have been standardized. This project has been transferred to the basic research area.

DD FORM 03 MAR 1498 EDITION OF MAR 60 IS OBSOLETE

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: INEQUALITY OF $\dot{V}A/\dot{Q}$ RATIOS FOLLOWING SMOKE INHALATION INJURY AND THE EFFECT OF ANGIOTENSIN ANALOGUES: Effects of (1-Sarcosin, 8-Isoleucine) Angiotensin II on Smoke Inhalation Injury

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Takeshi Shimazu, MD
George M. Vaughan, MD, Lieutenant Colonel, MC
Tetsuo Yukioka, MD*
Tsuyoshi Sugimoto, MD**
Gene B. Hubbard, DVM, MS, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

*Department of Emergency
and Critical Care Medicine
Tokyo Medical College
6-7-1, Hishi-Shinjuku
Shinjuku-ku, Tokyo, Japan

**Department of Traumatology
Osaka University Medical School
1-1-50 Fukushima, Fukushima-ku
Osaka, Japan

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: INEQUALITY OF $\dot{V}A/\dot{Q}$ RATIOS FOLLOWING SMOKE INHALATION INJURY AND THE EFFECT OF ANGIOTENSIN ANALOGUES: Effects of (1-Sarcosin, 8-Isoleucine) Angiotensin II on Smoke Inhalation Injury

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Takeshi Shimazu, MD
George M. Vaughan, MD, Lieutenant Colonel, MC
Tetsuo Yukioka, MD*
Tsuyoshi Sugimoto, MD**
Gene B. Hubbard, DVM, MS, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

*Department of Emergency
and Critical Care Medicine
Tokyo Medical College
6-7-1, Hishi-Shinjuku
Shinjuku-ku, Tokyo, Japan

**Department of Traumaology
Osaka University Medical School
1-1-50 Fukushima, Fukushima-ku
Osaka, Japan

REPORT CONTROL SYMBOL - MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: INEQUALITY OF $\dot{V}A/\dot{Q}$ RATIOS FOLLOWING SMOKE INHALATION INJURY AND EFFECTS OF ANGIOTENSIN ANALOGUES: Effects of (1-Sarcosin, 8-Isoleucine) Angiotensin II on Smoke Inhalation Injury

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: Takeshi Shimazu, MD
George M. Vaughan, MD, Lieutenant Colonel, MC
Tetsuo Yukioka, MD
Tsuyoshi Sugimoto, MD
Gene B. Hubbard, DVM, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

We have examined an angiotensin II analogue, (1-sarcosin, 8-isoleucine) angiotensin II, as a therapeutic agent in a sheep model of smoke inhalation injury. Twenty-four hours after smoke inhalation, baseline cardiopulmonary indices were measured under general anesthesia and under mechanical ventilation following two hours of stabilization. Then the angiotensin analogue was administered by continuous intravenous infusion for 30 minutes and the second measurements were made.

Six-hundred, 1,000, and 2,000 nanograms per kilogram body weight per minute doses were tested in 23 sheep with mild smoke inhalation injury and a 1,000 nanograms per kilogram body weight per minute dose was tested in seven normal uninjured sheep. The 1,000 nanograms per kilogram body weight per minute dose was also tested in another 21 sheep with various modes of injury, i.e., four sheep immediately after mild smoke inhalation injury, seven sheep 24 hours after moderate to severe injury, four sheep 24 hours after mild injury under spontaneous breathing, and six sheep 24 hours after carbon monoxide exposure. Plasma angiotensin-converting enzyme activity was measured to assess its diagnostic value and arterial and mixed-venous angiotensin I and angiotensin II levels were measured to evaluate the angiotensin system after smoke inhalation injury.

ABSTRACT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: INEQUALITY OF $\dot{V}A/\dot{Q}$ RATIOS FOLLOWING SMOKE INHALATION INJURY AND EFFECTS OF ANGIOTENSIN ANALOGUES: Effects of (1-Sarcosin, 8-Isoleucine) Angiotensin II on Smoke Inhalation Injury

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: Takeshi Shimazu, MD
George M. Vaughan, MD, Lieutenant Colonel, MC
Tetsuo Yukioka, MD
Tsuyoshi Sugimoto, MD
Gene B. Hubbard, DVM, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

REPORT CONTROL SYMBOL: MEDDH-288(R1)

We have examined an angiotensin II analogue, (1-sarcosin, 8-isoleucine) angiotensin II, as a therapeutic agent in a sheep model of smoke inhalation injury. Twenty-four hours after smoke inhalation, baseline cardiopulmonary indices were measured under general anesthesia and under mechanical ventilation following two hours of stabilization. Then the angiotensin analogue was administered by continuous intravenous infusion for 30 minutes and the second measurements were made.

Six-hundred, 1,000, and 2,000 nanograms per kilogram body weight per minute doses were tested in 23 sheep with mild smoke inhalation injury and a 1,000 nanograms per kilogram body weight per minute dose was tested in seven normal uninjured sheep. The 1,000 nanograms per kilogram body weight per minute dose was also tested in another 21 sheep with various modes of injury, i.e., four sheep immediately after mild smoke inhalation injury, seven sheep 24 hours after moderate to severe injury, four sheep 24 hours after mild injury under spontaneous breathing, and six sheep 24 hours after carbon monoxide exposure. Plasma angiotensin-converting enzyme activity was measured to assess its diagnostic value and arterial and mixed-venous angiotensin I and angiotensin II levels were measured to evaluate the angiotensin system after smoke inhalation injury.

A decrease in heart rate ($P < 0.01$), cardiac index ($P < 0.05$), and arterial carbon dioxide pressure ($P < 0.01$) and an increase in blood pressure ($P < 0.05$) and total peripheral resistance index ($P < 0.01$) were the significant changes caused by the angiotensin analogue for the overall groups ($n = 51$, paired t-test). These findings suggest that (1-sarcosin, 8-isoleucine) angiotensin II might be therapeutically useful in improving alveolar ventilation in smoke inhalation victims with stable hemodynamics.

ANGIOTENSIN I
ANGIOTENSIN II
ANGIOTENSIN-CONVERTING ENZYME ACTIVITY
CARDIOPULMONARY FUNCTION
SHEEP MODEL
SMOKE INHALATION INJURY
(1-SARCOSIN, 8-ISOLEUCINE) ANGIOTENSIN II

EFFECTS OF (1-SARCOSIN, 8-ISOLEUCINE) ANGIOTENSIN II ON SMOKE INHALATION INJURY

INTRODUCTION

Smoke inhalation injury is one of the primary determinants of survival following major burns. We have demonstrated that hypoxia induced by smoke inhalation is characterized by the development of low ventilation-perfusion compartments (1). Yukioka et al have examined the effects of angiotensin II (AII) analogues on acquired respiratory distress syndrome patients and oleic acid-induced lung injury in a sheep model and found that the angiotension II analogue improved pulmonary gas exchange (2-4). We have studied the AII analogue as a therapeutic agent following smoke inhalation injury and analyzed its cardiopulmonary effects in a sheep model.

Arterial and venous levels of angiotensins I and II were measured to elucidate the angiotensin system alterations after smoke inhalation and possibly correlate them with the changes induced by the AII analogue. We also measured plasma angiotensin-converting enzyme (ACE) activity after smoke inhalation to assess its diagnostic value as reported in some acute lung injuries (5-9).

---

[1]Shimazu T, Yukioka T, Hubbard GB, et al: A dose-responsive model of smoke inhalation injury: severity-related alteration in cardiopulmonary function. Ann Surg (in press).

[2]Yukioka T, Yukioka N, Aulick LH, et al: Evaluation of (1-sarcosin, 8-isoleucine) angiotensin II as a therapeutic agent for oleic acid-induced pulmonary edema. Surgery 99:235-244, 1986.

[3]Yukioka T, Sugimoto H, Yoshioka T, et al: Clinical application of (1-sar, 8-ile) angiotensin II for acute respiratory distress syndrome. Igaku Ayumi 123:168-170, 1982 (in Japanese).

[4]Yukioka T, Sawada Y, Sugimoto H, et al: Clinical study of (1-sar, 8-ile) angiotensin II as a therapeutic agent of ARDS. Geka Chir 46: 381, 1982 (in Japanese).

[5]Bedrossian CWM, Woo J, Miller WC, et al: Decreased angiotensin-converting enzyme in the adult respiratory distress syndrome. Am J Clin Path 70:244-247, 1978.

[6]Casey L, Krieger B, Kohler J, et al: Decreased serum angiotensin converting enzyme in adult respiratory distress syndrome associated with sepsis: a preliminary report. Crit Care Med 9:651-654, 1981.

MATERIALS AND METHODS

Animals. Fifty-one random source, neutered male sheep weighing 23 to 42 kilograms (31 ± 5.1 kilograms, mean standard deviation) were used for the study. The sheep were divided into six groups (Table 1):

Group 1. Seven healthy, uninjured sheep treated with AII analogue at 1,000 nanograms per kilogram body weight per minute (ng/kg/min).

Group 2A. Eight mildly injured sheep treated with AII analogue at 600 ng/kg/min.

Group 2B. Ten mildly injured sheep treated with AII analogue at 1,000 ng/kg/min.

Group 2C. Five mildly injured sheep treated with AII analogue at 2,000 ng/kg/min.

Group 3. Four sheep mildly injured and treated immediately after smoke exposure with AII analogue at 1,000 ng/kg/min.

Group 4. Seven moderately to severely injured sheep treated with AII analogue at 1,000 ng/kg/min.

Group 5. Four mildly injured sheep treated with AII analogue at 1,000 ng/kg/min under spontaneous breathing.

Group 6. Six sheep exposed to two-percent carbon monoxide gas mixture and treated with AII analogue at 1,000 ng/kg/min.

---

[7]Nukiwa T, Matsuoka R, Takagi H, et al: Responses of serum and lung angiotensin-converting enzyme activities in the early phase of pulmonary damage induced by oleic acid in dogs. Am Rev Respir Dis 126:1080-1086, 1982.

[8]Hollinger MA, Patwell SW, Zuckerman JE, et al: Effect of paraquat on serum angiotensin converting enzyme. Am Rev Respir Dis 121:795-198, 1980.

[9]Molteni A, Warpeha RL, Solliday NH, et al: The effect of burn and smoke inhalation on serum angiotensin-1-converting enzyme activity in humans (abstract). Bull Clin Rev Burn Inj 1(4):53, 1984.

TABLE 1

GROUPS AND STUDY DESIGN

Group	Number of Sheep	Injury	Time of Measurement After Injury	Ventilation	Angiotensin II Analogue Dose (ng/kg/min)
1	7	None	-	Mechanical	1,000
2A	8	Smoke Inhalation Injury/ Mild	24 Hours	Mechanical	600
2B	10	Smoke Inhalation Injury/ Mild	24 Hours	Mechanical	1,000
2C	5	Smoke Inhalation Injury/ Mild	24 Hours	Mechanical	2,000
3	4	Smoke Inhalation Injury/ Mild	Immediately After Smoke Exposure	Mechanical	1,000
4	7	Smoke Inhalation Injury/ Moderate to Severe	24 Hours	Mechanical	1,000
5	4	Smoke Inhalation Injury/ Mild	24 Hours	Spontaneous	1,000
6	6	Carbon Monoxide Exposure/ Moderate	24 Hours	Mechanical	1,000

Smoke Generation and Exposure. Sheep were exposed to smoke under general anesthesia as described previously (1,10). In Group 6, sheep were exposed to a gas mixture consisting of 12.6 percent oxygen, 5.2 percent carbon monoxide, 2.0 percent carbon monoxide, and 80.2 percent nitrogen, which simulates those concentrations in real smoke (11). The procedure of carbon monoxide exposure was the same as that for smoke exposure.

Measurements. Sheep were studied 24 hours after smoke exposure except for Group 3, in which measurements were made immediately after smoke exposure.

Following a two-hour stabilization period, cardiopulmonary indices were measured under general anesthesia and mechanical ventilation as described previously (1) except for Group 5, in which the animals were maintained in a sling and breathed spontaneously. Cardiopulmonary indices measured included blood pressure, heart rate, cardiac output, central venous pressure, pulmonary arterial pressure, pulmonary capillary wedge pressure, arterial and venous pH, partial oxygen pressure, partial carbon dioxide pressure, lung water, respiratory rate, tidal volume, flow rate, airway pressure, and end tidal partial oxygen and carbon dioxide pressure. (1-sarcosin, 8-isoleucine) AII (DD-3489F, Daiichiseiyaku, Tokyo, Japan) was then given intravenously for 30 minutes, at which time repeat measurements were made. The AII analogue was dissolved in 35 milliliters of normal saline solution and delivered at a constant rate of 0.12 milliliters per minute with a model 660-900 Harvard infusion pump (Model 660-900, Harvard Apparatus Company, South Natick, Massachusetts) (2).

Blood chemistry and hormonal measurements included a complete blood count, electrolytes, blood urea nitrogen, creatinine, calcium, total protein, plasma ACE, and arterial and venous angiotensins I and II. Plasma ACE activity was measured by ACE color test kit (Fujirebio Inc., Tokyo, Japan) distributed by Hana Biologics, Inc., Berkley, California) (12).

---

[10]Shimazu T, Yukioka T, McManus AT, et al: Inequality of $\dot{V}A/\dot{Q}$ ratios following smoke inhalation injury and the effect of angiotensin analogues: a large animal model of smoke inhalation injury with graded severity. In US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1984, pp 338-345.

[11]Shimazu T, Yukioka T, Hubbard GB, et al: Smoke inhalation injury and the effect of carbon monoxide in the sheep model (abstract). Proceedings of the Seventh International Congress on Burn Injuries, 23-28 February 1986, p A199.

[12]Kasahara Y and Ashihara Y: Colorimetry of angiotensin-1 converting enzyme activity in serum. Clin Chem 27:1922-1925, 1981.

Plasma angiotensin I and II were measured by radioimmunoassay (13).

Necropsies were performed on all sheep sacrificed at the end of the experiments. Data are shown as mean standard error. Comparison between groups for baseline data was made by analysis of variance. The effects of the AII analogue were analyzed by paired t-tests. Cardiopulmonary indices and changes of those indices by the AII analogue were correlated to the ACE and angiotensin levels by linear regression analysis (14). Differences were considered significant at $P < 0.05$.

RESULTS

Changes of cardiopulmonary indices are summarized in Table 2. A significant difference between groups for baseline data was found in the heart rate, blood pressure, partial oxygen pressure, partial carbon dioxide pressure, static compliance, pulmonary resistance, blood sugar, stroke volume index, and left ventricular stroke work index. These differences reflect the variety of groups especially those of spontaneous breathing (Group 5) and immediately after smoke (Group 3) and carbon monoxide exposure (Group 6) (11).

The effect of AII analogue was analyzed by a paired t-test. The decrease in heart rate, arterial carbon dioxide pressure, and cardiac index and the increase in blood pressure and total peripheral resistance index were significant for overall groups ($n = 51$). In Group 1, partial oxygen pressure and static compliance were significantly increased by the AII analogue and partial carbon dioxide was significantly decreased. In Group 2A, the increase in blood pressure and decrease in partial carbon dioxide pressure were significant. In Groups 2B and 3, the decrease in partial carbon dioxide pressure was significant. The increase in blood glucose level in Group 5 and increase in partial oxygen pressure in Group 6 were also significant. No other changes were significant in any group. In sheep measured under mechanical ventilation 24 hours after smoke exposure, i.e., Groups 2A, 2B, 2C, and 4 combined ($n = 30$), the decrease in heart rate and arterial carbon dioxide pressure and the increase in pulmonary resistance and total peripheral resistance index were significant.

---

[13] Shirani KZ, Vaughan GM, Lehrner LM, et al: Studies of the neuroendocrine abnormalities in burn injury: changes in the renin-angiotensin-aldosterone axis of burn patients. In US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1983. 74-120, 1983.

[14] Dixon WJ (ed). BMDP Statistical Software. Berkeley: University of California, 1983.

TABLE 2

SELECTED CARDIOPULMONARY INDICES BEFORE AND AFTER ANGIOTENSIN II ANALOGUE ADMINISTRATION (MEAN ± STANDARD ERROR)

Group	Heart Rate (Beats/Minute)	Blood Pressure (Torr)	Cardiac Index (Liter/Minute)	Total Peripheral Resistance Index (dynes sec m^2/cm^{-5})	Arterial Oxygen Pressure (Torr)	Arterial Carbon Dioxide Pressure (Torr)
1	171 ± 11	113 ± 4	3.5 ± 0.3	1972 ± 234	86 ± 5	28.1 ± 1.4
	167 ± 10	114 ± 5	3.4 ± 0.3	2790 ± 249	92 ± 4*	26.7 ± 1.5*
2A	164 ± 9	122 ± 7	3.7 ± 0.5	2728 ± 281	82 ± 5	31.2 ± 2.0
	153 ± 11	128 ± 7*	3.4 ± 0.4	3214 ± 341	85 ± 4	29.9 ± 1.7*
2B	157 ± 5	116 ± 3	4.2 ± 0.3	2255 ± 276	76 ± 3	29.0 ± 0.5
	149 ± 6	115 ± 4	3.9 ± 0.3	2395 ± 167	76 ± 3	28.1 ± 0.5*
2C	161 ± 11	105 ± 2	3.1 ± 0.3	2735 ± 276	82 ± 4	26.1 ± 1.0
	162 ± 9	105 ± 3	3.1 ± 0.3	2796 ± 228	79 ± 8	25.9 ± 1.1
3	174 ± 17	115 ± 7	3.8 ± 0.6	2529 ± 365	65 ± 6	31.0 ± 2.1
	165 ± 21	119 ± 6	3.5 ± 0.5	2853 ± 401	69 ± 6	29.1 ± 2.1*
4	177 ± 16	111 ± 8	3.6 ± 0.4	2564 ± 249	49 ± 4	33.1 ± 3.2
	162 ± 14	113 ± 9	3.5 ± 0.5	2705 ± 269	47 ± 4	33.6 ± 3.2
5	102 ± 13	98 ± 7	4.2 ± 0.7	1972 ± 234	66 ± 11	34.4 ± 1.1
	101 ± 11	105 ± 6	4.8 ± 0.7	1803 ± 176	62 ± 11	34.5 ± 1.1
6	170 ± 5	132 ± 16	4.6 ± 0.4	2377 ± 312	82 ± 3	30.1 ± 1.1
	158 ± 8	131 ± 5	4.1 ± 0.2	2592 ± 227	88 ± 2*	29.3 ± 1.0
TOTAL	161 ± 4	114 ± 2	3.8 ± 0.2	2473 ± 88	74 ± 2	30.2 ± 0.7
	153 ± 4	117 ± 2	3.7 ± 0.1	2672 ± 101	76 ± 3	29.5 ± 0.7
	P < 0.01	P < 0.05	P < 0.05	P < 0.01	NS	P < 0.05

*P < 0.05.
**P < 0.01.

NOTE: Numbers on the upper line are baseline values and those on the lower line are values 30 minutes after angiotensin II analogue administration.

Infusion of AII analogue always caused a transient elevation in systemic blood pressure. However, pulmonary artery pressure did not increase and, in fact, decreased slightly in some cases. A typical example of these changes are shown in Figure 1.

Baseline values of the ACE and angiotensins are summarized in Table 3. Angiotensin levels were measured only in half of the cases (n = 27). A significant difference between groups was not found in these measurements. Angiotensin conversion indices showed no significant differences between groups.

The correlations between arterial and venous levels of angiotensins I and II were analyzed by linear regression (Table 4). A moderate to good correlation (correlation coefficient ranging from 0.59 to 0.96) was found.

Correlations between cardiopulmonary indices and the ACE or angiotensins were also assessed by linear regression analysis. Significant correlation ($P < 0.05$) was found between the ACE and blood pressure, AII and blood pressure, AII and arterial oxygen pressure, and AII and arterial carbon dioxide pressure (Table 5).

Changes in cardiopulmonary indices (difference between pre- and post-values) induced by AII analogue infusion were correlated with the baseline levels of angiotensin I, AII, or angiotensin-conversion index 2 by linear regression analysis, but no significant correlation was found.

DISCUSSION

We have studied alterations in plasma angiotensin levels and cardiopulmonary indices following smoke inhalation injury and evaluated (1-sarcosin, 8-isoleucine) AII as a therapeutic agent for smoke inhalation injury. Angiotensin I and II levels after smoke inhalation injury were not significantly different from those in control sheep. This might be attributable in part to the wide range of control levels and in part to the lack of angiotensin measurements in the severely injured group (Group 4), in which pulmonary circulation sustained greater damage and a larger change in AII conversion would be expected (1,15). Conversion of angiotensin I in the lung to AII was reflected by only a small elevation of arterial AII from venous level and conversion indices (Table 3) did not differ among groups. Although we cannot directly compare the values we obtained to the reported values of AII in spontaneously

---

[15]Fanburg BL and Glazier JB: Conversion of angiotensin 1 to angiotensin 2 in the isolated perfused dog lung. J Appl Physiol 35:325-331, 1973.

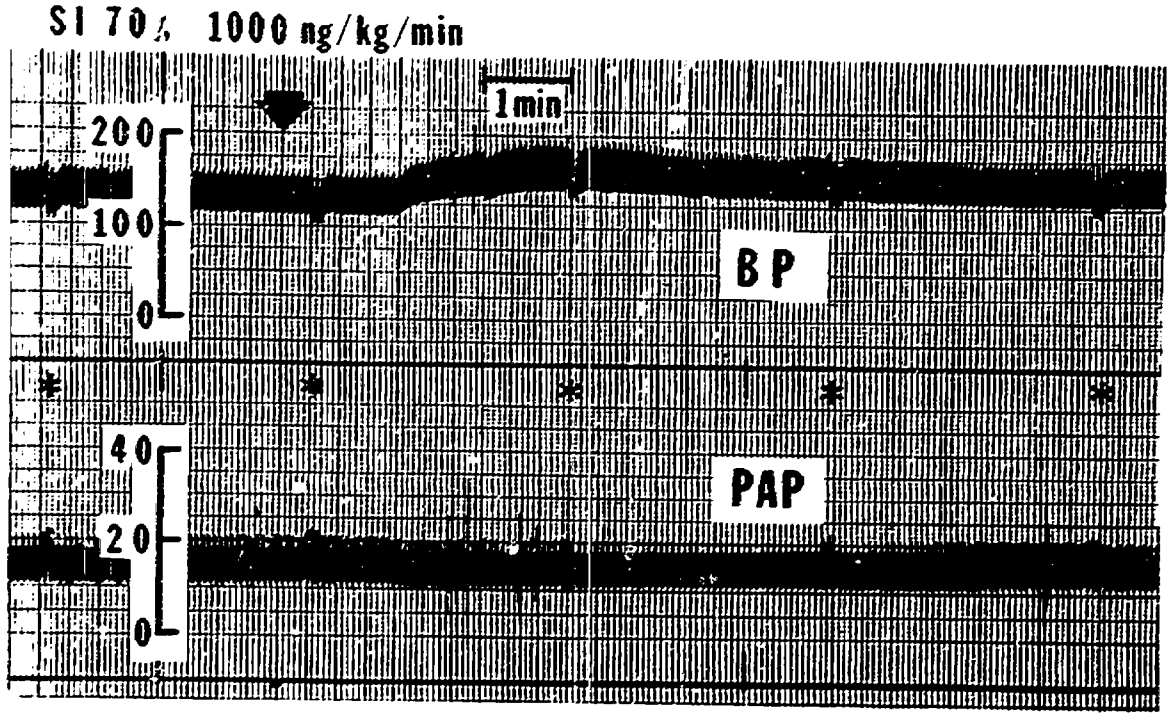


FIGURE 1. Typical example of transient arterial blood pressure elevation after infusion of (1-sarconsin, 8-isoluecine) angiotensin II. The arrow indicates start of the angiotensin II analogue infusion. The asterisk indicates sigh ventilation at a three-minute interval.

TABLE 3

ANGIOTENSIN-CONVERTING ENZYME, ANGIOTENSIN I, AND ANGIOTENSIN II FOR EACH GROUP (MEAN ± STANDARD ERROR)

	ACE	AIA	AIV	AIIA	AIIV	ACI-1	ACI-2
Group 1 (n = 7)	3.2 ± 0.3	379 ± 115	380 ± 127	56 ± 24	45 ± 22	1.34 ± 0.17	1.30 ± 0.11
Group 2 (n = 9)	3.7 ± 0.3	522 ± 238	471 ± 167	36 ± 8	28 ± 5	1.31 ± 0.14	1.37 ± 0.19
Group 3 (n = 3)	4.5 ± 0.2	902 ± 343	937 ± 320	118 ± 58	102 ± 48	1.17 ± 0.07	1.31 ± 0.18
Group 4 (n = 6)	4.1 ± 0.5	-	-	-	-	-	-
Group 5 (n = 4)	3.6 ± 0.3	424 ± 193	450 ± 203	265 ± 206	70 ± 32	1.25 ± 0.03	1.36 ± 0.03
Group 6 (n = 4)	4.6 ± 0.9	525 ± 124	546 ± 105	58 ± 20	47 ± 13	1.20 ± 0.10	1.26 ± 0.03

NOTE: ACE = angiotensin-converting enzyme, AIA = arterial angiotensin I (picograms per milliliter), AIV = venous angiotensin I, AIIA = arterial angiotensin II (picograms per milliliter), AIIV = venous angiotensin II, ACI-1 = angiotensin conversion index 1 (AIIA/AIIV), ACI-2 = angiotensin conversion index 2 (AIV X AIIA)/(AIA X AIIV). No significant differences between groups were found by analysis of variance.

TABLE 4

CORRELATION BETWEEN ARTERIAL AND VENOUS ANGIOTENSINS I AND II

	Number of Sheep	Correlation Coefficient	P Value
Arterial Angiotensin I and Venous Angiotensin I	27	0.958	< 0.001
Arterial Angiotensin II and Venous Angiotensin II	26	0.643	< 0.001
Arterial Angiotensin I and Arterial Angiotensin II	26	0.602	< 0.001
Venous Angiotensin I and Venous Angiotensin II	27	0.774	< 0.001
Arterial Angiotensin I and Venous Angiotensin II	27	0.722	< 0.001
Venous Angiotensin I and Arterial Angiotensin II	26	0.587	< 0.01

TABLE 5

CORRELATION BETWEEN ANGIOTENSIN II AND CARDIOPULMONARY INDICES

	Number of Sheep	Correlation Coefficient	P Value
Angiotensin-Converting Enzyme (X) and Blood Pressure (Y)	36	0.436	< 0.05
Arterial Angiotensin II (X) and Blood Pressure (Y)	26	-0.550	< 0.05
Arterial Angiotensin II (X) and Partial Oxygen Pressure (Y)	26	-0.564	< 0.05
Arterial Angiotensin II (X) and Partial Carbon Dioxide Pressure (Y)	26	0.584	< 0.05
Venous Angiotensin II (X) and Blood Pressure (Y)	27	-0.507	< 0.05
Venous Angiotensin II (X) and Partial Oxygen Pressure (Y)	27	-0.535	< 0.05
Arterial Angiotensin II (X) and Partial Carbon Dioxide Pressure (Y)	27	0.541	< 0.05

breathing sheep from other laboratories (10 to 20 picograms per milliliter) (16), our control group sheep had higher levels of AII (Table 3), which might be explained by the anesthesia used in our experiments. Other factors that might have affected the results are that sheep seem to have (5-Val) AII instead of (5-isoleucine) AII which is present in human and pig blood and that the antibody to AII reacts not only with the octapeptide (AII) but also with its fragments, such as hepta- and hexa-peptides. The antibody we used in this study was developed for human AII (13), but a change at the fifth amino acid seems to have little effects on its antigenicity. Thus, dilutions of sheep serum produced changes in binding parallel to those of the standard curve (using human AII, data not shown), the levels obtained were similar to those measured in humans, and the negative correlation of AII values in this study with blood pressure (Table 5) suggests an appropriate physiologic response of AII as measured herein to variations in blood pressure. A difference in one amino acid might have resulted in lower reactivity to antibody, but it would not have falsely increased the level of immunologically measured AII.

In a previous study, we observed an injury severity-related response in blood pressure and arterial oxygen pressure (10). In this study, baseline levels of AII showed moderate negative correlation with blood pressure, arterial oxygen pressure and arterial carbon dioxide pressure (Table 5). If the elevated AII levels contributed to impaired gas exchange by altering pulmonary circulation or airway tension through direct vasoconstrictive action or sympathomimetic action, AII analogues (if it is antagonistic) could be therapeutically applied to improve ventilation and oxygenation.

(1-sarcosin, 8-isoleucine) AII significantly decreased heart rate, arterial carbon dioxide pressure, and cardiac index and increased blood pressure and total peripheral resistance index for the overall groups (Table 2). Similar changes (heart rate, arterial carbon dioxide pressure, pulmonary resistance, and total peripheral resistance index) were significant in smoke-exposed sheep measured under mechanical ventilation 24 hours after exposure (Groups 2 and 4 combined, n = 30, Table 2), which simulate smoke inhalation patients in an intensive care unit setting. Although we could not draw conclusion on dose response of the AII analogue because of the smaller number of Group 2C sheep, significant reduction of arterial carbon dioxide pressure in Group 2A (600 ng/kg/min) and Group 2B (1 000 ng/kg/min) and concomitant elevation of blood pressure

---

[16]Cain MD, Catt KJ, Coghlan JP, et al: Evaluation of angiotensin II metabolism in sheep by radioimmunoassay. Endocrinology 86:955-964, 1970.

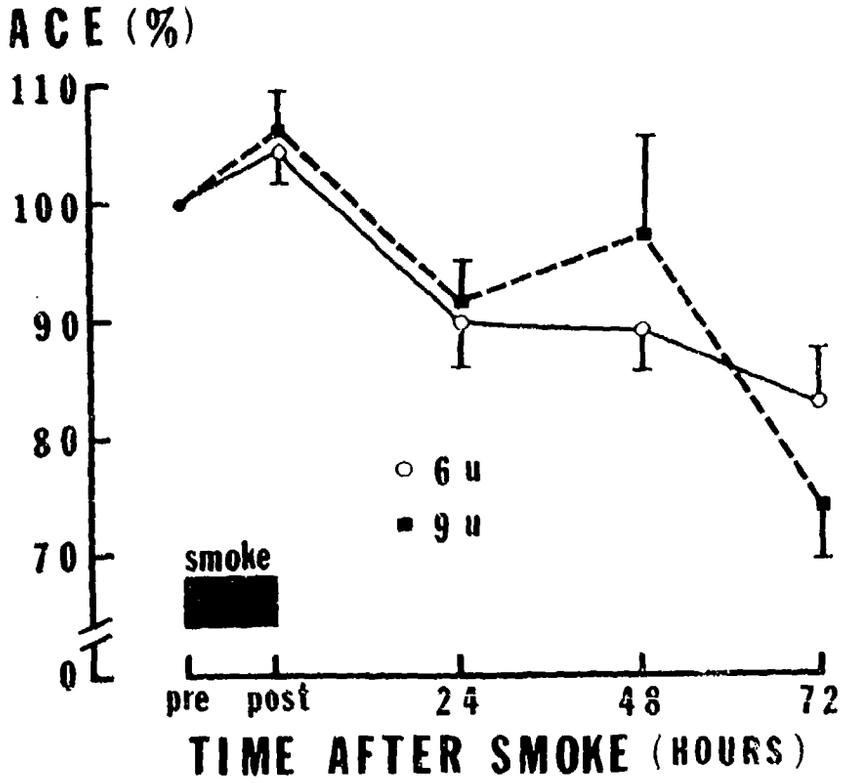


FIGURE 2. Plasma angiotensin-converting enzyme levels after different extents of smoke inhalation injury (mean standard error). Angiotensin-converting enzyme activity is expressed as a percentage of the value before smoke exposure. Open circles indicate a six-unit exposure (mild to moderate injury) and closed circles indicate a nine-unit exposure (moderate to severe injury).

in Group 2A suggest that the AII analogue improved alveolar ventilation and 1,000 ng/kg/min dose would be recommended because blood pressure did not show significant change at that dose. Yukioka et al reported improved oxygenation as well as increased alveolar ventilation by the AII analogue in acquired respiratory distress syndrome patients and am animal model of oleic acid-induced injury, but we did not observe improved oxygenation in smoke-exposed sheep in this study (2-4). Increased oxygenation, however, was seen only in controls and the sheep exposed to carbon monoxide, in which no anatomical damage to the lung was seen (Table 2) (11). The mechanism of

the airway effect of the AII analogue is not clear; it increased alveolar ventilation, increased airway resistance, but an increase in oxygenation was not a constant finding. The increased total peripheral resistance index, however, suggests that the AII analogue exerted vasoconstrictive (agonistic) action to the systemic vasculature as Hata et al reported (Figure 1) (17) and might further compromise circulation in hemodynamically unstable patients.

Blood ACE activity has been said to be a marker of pulmonary capillary endothelial damage and an useful index for diagnosing acquired respiratory distress syndrome and other acute lung injury (6-8). There are also several reports that deny its diagnostic value (18-19). Traber et al reported increased blood ACE activity in an ovine model of smoke inhalation injury and Molteni et al reported increased blood ACE activity in burn patients with smoke inhalation, but the statistical significance of those changes was not identified (9,20). In this study, we did not observe significant change in ACE activity 24 hours after smoke exposure (Table 2). Figure 2 shows the changing ACE activity after smoke exposure in mild to moderate (six units) and moderate to severe (nine units) injuries observed in a previous study (1). There was a slight increase immediately after smoke exposure and then a decrease over the next 72 hours. This trend became clear only after expressing the changes as percent change from the baseline level. Judging from this observation and the relatively wide normal range of ACE activity (21), blood ACE activity measurements would appear to be of little use in diagnosing smoke inhalation injury.

---

[17]Hata T, Ogihara T, Mikami H, et al: Effects of two angiotensin II analogues on blood pressure, plasma aldosterone concentration, plasma renin activity, and creatinine clearance in normal subjects on different sodium intakes. Eur J Clin Pharmacol 18:295-299, 1980.

[18]Fourrier F, Chopin C, Wallaert B, et al: Angiotensin-converting enzyme in human adult respiratory distress syndrome. Chest 83:593-597, 1983.

[19]Krieger B, Schwartz J, Loomis W, et al: Nonspecificity of elevated angiotensin-converting enzyme activity in bronchoalveolar lavage fluid from high permeability lung edema status. Am Rev Respir Dis 129:499-500, 1984.

[20]Traber D, Schlag G, Redi H, et al: The mechanism of the pulmonary edema of smoke inhalation injury (abstract). Circulatory Shock 13:77, 1984.

[21]Turton CW, Grundy E, Firth G, et al: Value of measuring serum angiotensin 1 converting enzyme and serum lysozyme in the management of sarcoidosis. Thorax 34:57-62, 1979.

In conclusion, (1-sarcosin, 8-isoleucine) AII improved alveolar ventilation following smoke inhalation injury and produced slight increase in cardiac afterload. This agent might be of use in smoke inhalation patients with hypercapnea and stable hemodynamics. The 1,000 ng/kg/min dose seems to be better than the 600 or 2,000 ng/kg/min doses. Plasma levels of AII were not dramatically altered 24 hours after mild smoke inhalation nor did they predict a response to the analogue. Blood ACE activity appeared to be of no diagnostic significance for smoke inhalation injury.

PUBLICATIONS/PRESENTATIONS

None.

ACKNOWLEDGMENT

The authors gratefully acknowledge Craig Daniel for his assistance in measuring ACE activity during his senior elective course.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: INEQUALITY OF $\dot{V}A/\dot{Q}$ RATIOS FOLLOWING SMOKE INHALATION INJURY AND THE EFFECT OF ANGIOTENSIN ANALOGUES: Platelet-Activating Factor (PAF) in a Sheep Model of Smoke Inhalation Injury - A Preliminary Report

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Takeshi Shimazu, MD
Hisashi Ikeuchi, MD
Raj Kumar, PhD*
Donald J. Hanahan, PhD*
Gene B. Hubbard, DVM, MS, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

*Department of Biochemistry
The University of Texas
Health Science Center
San Antonio, Texas

ABSTRACT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: INEQUALITY OF $\dot{V}A/\dot{Q}$ RATIOS FOLLOWING SMOKE INHALATION INJURY AND EFFECTS OF ANGIOTENSIN ANALOGUES: Platelet-Activating Factor (PAF) in a Sheep Model of Smoke Inhalation Injury - A Preliminary Report

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: Takeshi Shimazu, MD
Hisashi Ikeuchi, MD
Raj Kumar, PhD
Donald J. Hanahan, PhD
Gene B. Hubbard, DVM, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

PAF is a recently identified chemical mediator and is considered to participate in many allergic and inflammatory responses. Its pathophysiologic activity includes bronchoconstriction and vasopermeability change which are both observed in smoke inhalation injury. We have studied the possible imvolvement of PAF as a key mediator in smoke inhalation injury. Six chronically instrumented sheep were given a standard smoke inhalation injury. Blood, pulmonary lymph, and bronchoalveolar lavage fluid were collected for analysis of PAF prior to smoke and one, six, 12, and 24 hours after smoke exposure. PAF was not found in any blood or lymph samples. PAF was positive in the bronchoalveolar lavage fluid from all but one sheep. Among the five sheep that were PAF-positive, one showed PAF in the presmoke sample and other sheep became positive at one hour after smoke exposure while other positive specimens were taken only at six hours after smoke exposure or later. The level of PAF increased with time in five sheep, including the two sheep which showed PAF at presmoke and one hour after exposure. Pulmonary lymph flow increased with time, but changes in arterial oxygen pressure and cardiac output were not related either to time or to the level of PAF.

Thick pseudomembrane was observed in all the sheep at necropsy, including the four that died by 24 hours, but PAF was not always present in the last bronchoalveolar lavage samples taken at regular interval before death. Progressive inflammatory response was always observed by six hours after smoke exposure, even in milder smoke inhalation injury in previous studies, but PAF in the bronchoalveolar lavage fluid was not always positive at six hours in this study (positive in two, trace in one, and negative in two). These results suggest that PAF only participated in the inflammatory process and did not trigger inflammatory response. Although this is a very limited study, it could at least be concluded that PAF did not satisfy requirements to be the key chemical mediator, if any, of smoke inhalation injury.

PLATELET-ACTIVATING FACTOR (PAF)
SMOKE INHALATION INJURY
SHEEP MODEL
LUNG LYMPH FLUID
BRONCHALVEOLAR LAVAGE

PLATELET-ACTIVATING FACTOR (PAF) IN A SHEEP MODEL OF SMOKE INHALATION INJURY - A PRELIMINARY REPORT

INTRODUCTION

Smoke inhalation injury significantly increases mortality in patients with major burns. The role of chemical mediators in the pathophysiologic changes of smoke inhalation injury has been studied to identify effective antagonists or inhibitors (1-4). Pathophysiologic changes that occur after smoke inhalation injury include activation of neutrophils, bronchoconstriction, and increased pulmonary capillary permeability (5). PAF (or acetyl glyceryl ether phosphorylcholine) has been reported to evoke many allergic and inflammatory responses and produce cardiopulmonary alterations, vasopermeability alterations, smooth muscle contraction, and leukocyte activation as well as platelet activation (6-7). Those actions suggest that PAF might be an important chemical mediator in smoke inhalation injury. In this study, we have measured PAF in blood, lung lymph, and bronchoalveolar lavage fluid obtained from smoke insufflated sheep to study its possible imvolvement in the progressive respiratory insufficiency characteristic of smoke inhalation injury.

---

[1]Stein MD: Boyden chamber analysis of sheep neutrophil chemotaxis (abstract). Circ Shock 13:77-78, 1984.

[2]Stein MD, Herndon DN, Stevens JM, et al: Production of chemotactic factors and lung cell changes following smoke inhalation in a sheep model. JBCR 7:117-121, 1986.

[3]Desai MH, Brown M, Mlcak R, et al: Reduction of smoke-induced lung injury with dimethylsulfoxide and heparin treatment. Surg Forum 36:103-104, 1985.

[4]Stewart R, Yamaguchi K, Rowland R, et al: Pulmonary edema formation following smoke inhalation and cimetidine injection using a rabbit model and gamma imaging techniques (abstract 145). Proceedings of the Eighteenth Annual Meeting of the American Burn Association, 1986.

[5]Stephenson SF, Esrig BC, Polk HC, et al: The pathophysiology of smoke inhalation injury. Ann Surg 182:652-660, 1975.

[6]Chignard M, Le Couedic TP, Tence M, et al: The role of platelet-activating factor in platelet aggregation. Nature 279:799-800, 1979.

[7]Linda M, McManus R, Pinckard N, et al: Acetyl glyceryl ether phoshorylcholine (AGEPC) in allergy and inflammation. In Skandia International Symposia, Theoretical and Clinical Aspects of Allergic Diseases. Stockholm: Almqvist & Wiksell International, 1983, p 165-182.

MATERIALS AND METHODS

Six female sheep weighing 43 to 51 kg (46.7 2.7 kilograms) were used in this study. The sheep were operated for lung lymph cannulation and arterial and Swan-Ganz catheter placement three to five days before smoke exposure. Tracheostomy was performed one to three days before smoke exposure. The sheep were insufflated with smoke, which produced mild to moderate injury in previous studies (8). Blood, lung lymph, and bronchoalveolar lavage samples were taken prior to smoke and one, six, twelve, and 24 hours after smoke. Blood gas, blood and lymph chemistries, and hemodynamic measurements were made at the same times. Each sheep was kept under spontaneous breathing in a metabolic cage throughout the experiment, except during the smoke insufflation procedure which was carried out under general anesthesia (8).

PAF extraction from blood was performed according to the method described by Pinckard et al (9). Since PAF is catabolized very rapidly by acetylhydrolase, which is abundant in plasma, acetylhydrolase was inactivated by acidifying the blood below pH 3 immediately after sampling. Recovery of exogenous H3-labelled PAF by this method is approximately 50 percent. Acidification by hydrogen chloride alone causes hemolysis of sheep blood and altered retardation factor in thin-layer chromatography. To minimize hemolysis by acidification, 0.3N hydrogen chloride diluted by acid-citrate-dextrose (USP, Formula A) was used. After centrifugation, the plasma was separated and mixed with methanol and chloroform. Following 30-minutes incubation at room temperature, the blood-methanol-chloroform mixture was centrifuged and the methanol phase separated. A second extraction was performed by using chloroform and water. The chloroform layer was collected for PAF analysis. Lymph (one milliliter) and lavage (two to five milliliters) samples were treated similarly except for the omission of the initial acidification process, since there is little acetylhydrolase in those fluids. The extracts were isolated and purified by

---

[8]Shimazu T, Yukioka T, Hubbard GB, et al: A dose-responsive model of smoke inhalation injury: severity-related alteration in cardiopulmonary function. Ann Surg (in press).

[9]Pinckard RN, Farr RS, and Hanahan DJ: Physicochemical and functional identity of rabbit platelet-activating factor (PAF) released in vivo during IgE anaphylaxis with PAF released in vitro from IgE sensitized basophils. J Immunol 123:1847-1857, 1979.

thin-layer chromatography (9-10). PAF activity was assayed on rabbit platelets by the aggregation method (10). Necropsies were performed on all sheep.

RESULTS

Two sheep survived 24 hours and the others died between five and 20 hours after smoke exposure. All sheep developed thick pseudomembranes on the major airways, which partially occluded the lumen (Figure 1). The peak carboxyhemoglobin level after smoke exposure averaged 54.8 ± 5.2 percent (mean ± standard deviation). This level was consistant with the carboxyhemoglobin levels of mild injury in previous studies, but the pseudomembrane formation and mortality in this study was disproportionately severe. This may be attributable to the drying of the airway due to the tracheostomy.

PAF was not found in any blood or lung lymph samples. PAF was positive in the bronchoalveolar lavage fluid in five sheep, including two showing only trace level (Table 1). One sheep showed PAF in the presmoke sample, became negative at one hour, and increased with time after six hours. Another sheep showed a similar increase with time after one hour. The other three sheep did not show an increase with time, but became PAF-positive only at six hours or later. The sheep in which PAF was not found died at five hours after smoke inhalation injury. This sheep is supposed to have sustained severest injury among the six sheep studied, but PAF was not found in the one hour sample.

Changes in the arterial oxygen pressure are shown in Figure 2. The arterial oxygen pressure decreased significantly after smoke exposure (one hour) and recovered to subnormal levels by six hours. Thereafter surviving sheep showed a slow decrease in arterial oxygen pressure. One of the sheep had a lower baseline arterial oxygen pressure value and showed severe respiratory distress after smoke insufflation. This sheep was ventilated mechanically from 15 minutes after smoke exposure with fractional inspired oxygen of 0.4 (indicated by the asterisk in Figure 2). The arterial oxygen pressure of this sheep decreased progressively after one hour and the animal died at 11 hours after smoke exposure.

Changes in the cardiac index are shown in Figure 3. The cardiac index increased after smoke inhalation to compensate for the loss of available hemoglobin due to carboxyhemoglobin

---

[10]Hanahan DJ: Platelet-activating factor isolation, identification, and assay. In Glick D (ed). Methods of Biochemical Analysis, Volume 31. New York: John Wiley & Sons, Inc., 1985, pp 195-219.

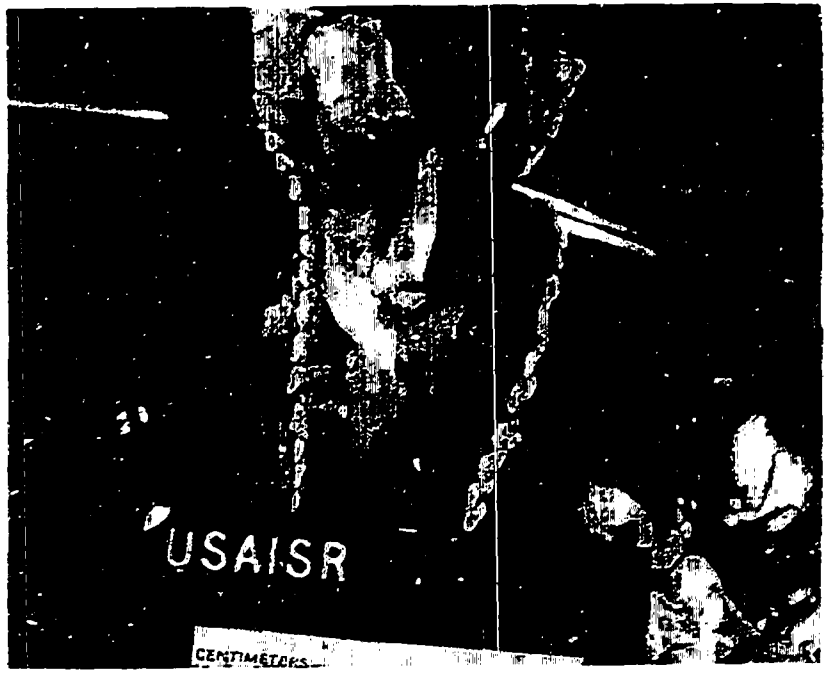


FIGURE 1. Pseudomembrane formation on the trachea, partially occluding the lumen.

TABLE 1

PAF IN BRONCHOALVEOLAR LAVAGE FLUID (PICOMOLE PER SAMPLE)

Sheep Number	Pre-Smoke	Post-Smoke	Post-Smoke	Post-Smoke	Post-Smoke
1	-	-	Trace	Dead	
2	0.14	-	0.24	0.46	0.67
3	-	-	-	Trace	Dead
4	-	-	-	-	1.75
5	-	1.90	3.48	3.35	1.75
6	-	-	Dead		

Trace = Aggregation only.

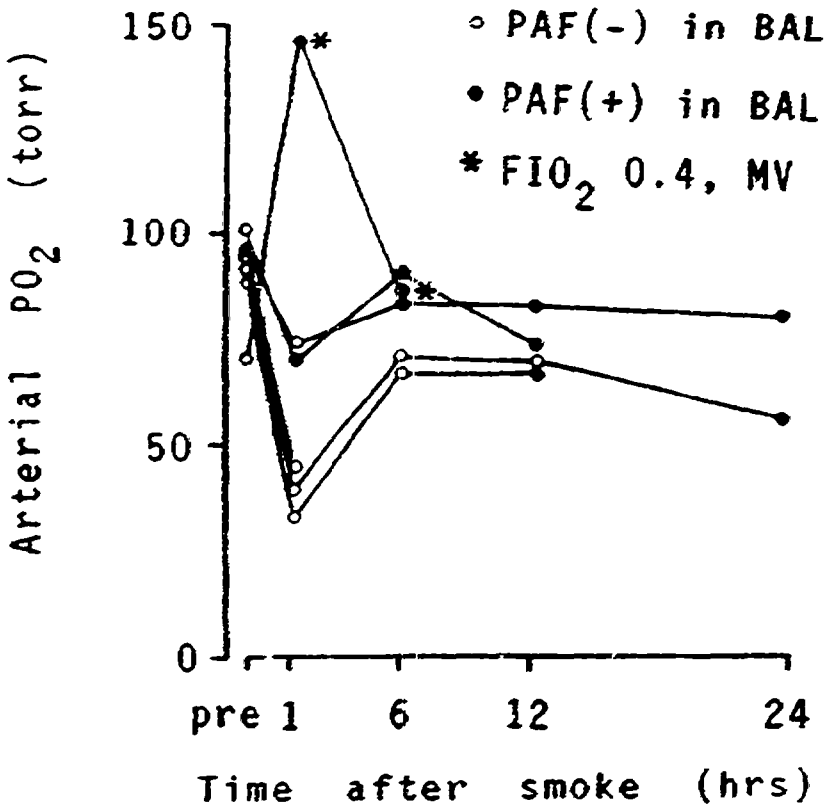


FIGURE 2. Changes in arterial oxygen pressure. Closed circles indicate samplings at which time PAF was positive in the bronchoalveolar lavage fluid. The asterisk indicates Sheep #1 which developed severe respiratory distress soon after smoke exposure and was mechanically ventilated with fractional inspired oxygen of 0.4.

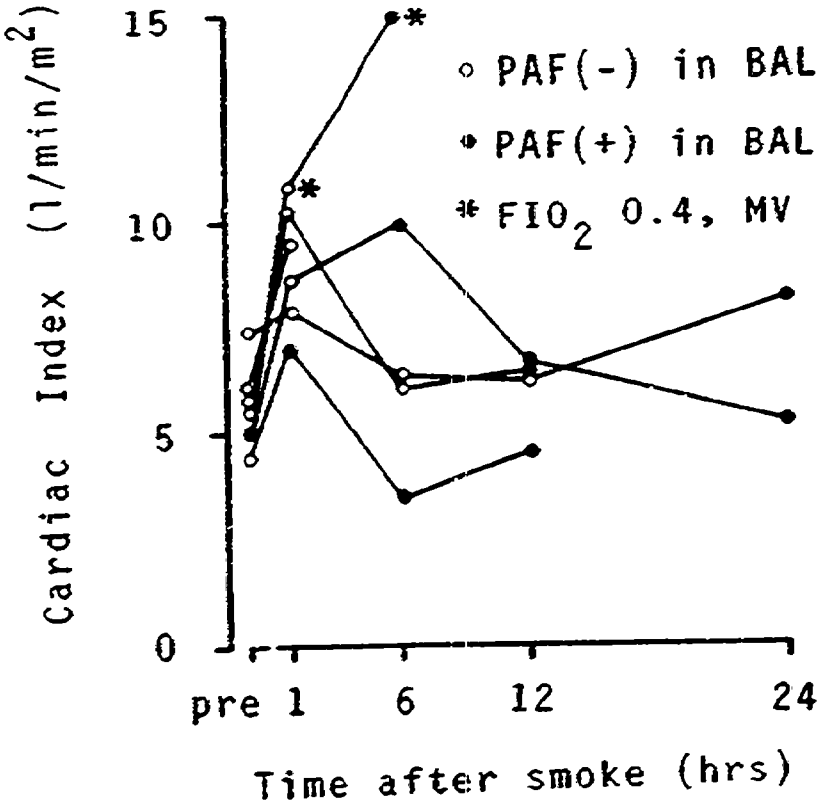


FIGURE 3. Changes in cardiac index.

formation. After six hours, variable changes in the cardiac index were noted in each sheep. The sheep maintained under mechanical ventilation showed a progressive increase in the cardiac index with time.

Changes in lymph flow are shown in Figure 4. The lymph cannulae remained patent throughout the experiment in four sheep. In three sheep out of the four, lymph flow was not increased at one hour after exposure. Thereafter it increased at a variable rate in each sheep. The three sheep in which lymph flow increased above 10 milliliters per hour within the first six hours postinjury all died within 24 hours.

DISCUSSION

Acetylhydrolase activity was measured in a few lymph samples. Activity of acetylhydrolase was measured by incubating the specimen with a known amount of PAF and measuring the remaining PAF activity after incubation. Lymph fluid from the thoracic duct showed high activity of acetylhydrolase, while lymph fluid from lung lymph duct (efferent duct from the caudal mediastinal de) showed no activity. The lipid content in lung lymph flu . was much less than that in thoracic duct lymph. This suggests that lung lymph fluid is a good source for PAF detection, although the volume available for study is limited by low lung lymph flow rates (normal = four to six milliliters per hour).

Many studies of exogenous PAF administration or PAF inhibitors have been carried out, but studies of disease conditions in which endogenous PAF has been assayed are few (11). Involvement of PAF in acute lung injuries, like endotoxin shock, has been reported on the basis of the effects of a specific PAF antagonist (12); the evidence is still indirect. We have measured PAF from blood, lung lymph fluid, and bronchoalveolar lavage fluid and found PAF only in the bronchoalveolar lavage fluid. Recovery of PAF from the blood was, as mentioned above, relatively low. Lung lymph fluid, although the volume of the specimens is limited, should be a good material for assay of PAF (because acetylhydrolase activity was negligible) to see if PAF is responsible for lung edema formation after smoke inhalation. However, we could not detect PAF in the lung lymph fluid in these studies.

---

[11]Caramelo C, Fernandez-Gallardo S, Marin-Cao D, et al: Presence of platelet-activating factor in blood from humans and experimental animals. Its absence in anephric individuals. Biochm Biophysic Res Commun 120:789-796, 1984.

[12]Page CP and Robertson DN: PAF in shock and lung injury. In A NATO Advanced Research Workshop on Lipid Mediators in Immunology of Burn and Sepsis, Volume 39. Helsingor, 1986.

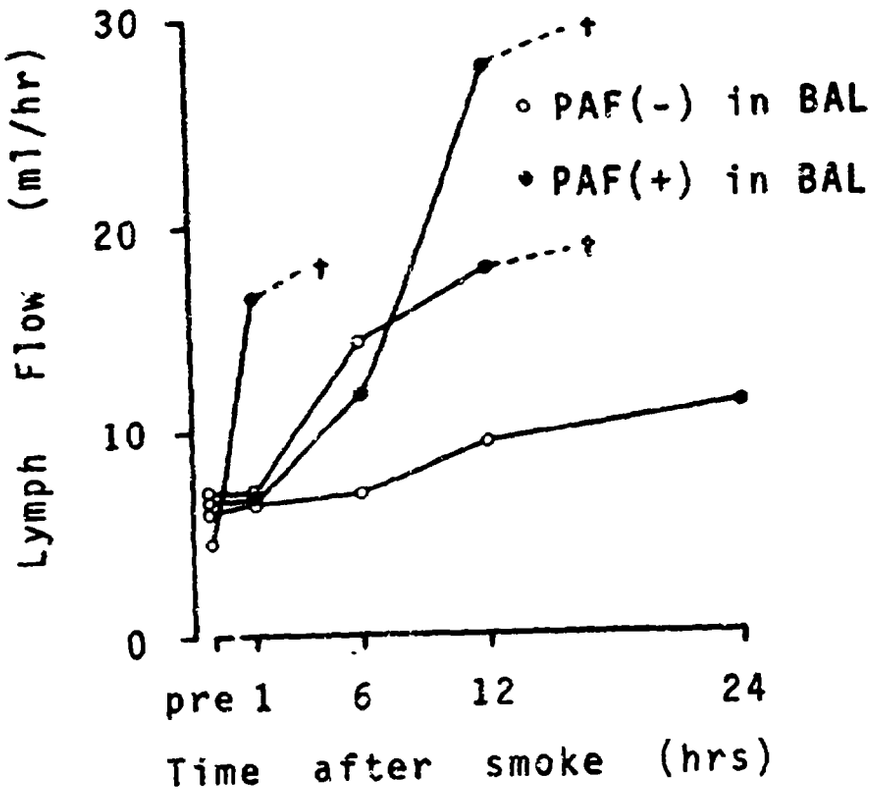


FIGURE 4. Changes in lung lymph flow. The lung lymph cannula remained patent throughout the study in only four of the six sheep.

In a previous study, we examined changes in histologic findings serially after smoke inhalation. There was minimal change in trachea and the lung parenchyma at one hour after smoke exposure, while an inflammatory response was always evident by six hours, even in milder smoke inhalation injuries. This suggests that appearance of PAF in the bronchoalveolar lavage fluid does not preceed inflammatory response and that PAF is not the initial mediator. PAF does appear to be involved in the inflammatory process evoked by smoke inhalation injury since PAF was detected in five sheep and two of them showed a typical increase with time. Specific PAF antagonists may ammeliorate the pathophysiologic consequence of the inflammation produced by smoke inhalation injury (13).

In summary, we measured PAF after smoke inhalation injury in blood, lung lymph fluid, and bronchoalveolar lavage fluid. PAF was found only in the bronchoalveolar lavage fluid, but it does not appear to be a component of the early phase of imflammatory response. This suggests that PAF is not a primary chemical mediator in smoke inhalation injury.

ACKNOWLEGEMENT

The authors gratefully acknowledge Dr. Kunio Miwa of the Biochemistry Department at the University of Texas Health Science Center at San Antonio, Texas, for his helpful advices and cooperation.

[13]Braquet P, Touqui L, Bargaftig BB, et al: Perspectives in platelet activating factor research. J Med Chem (in press).

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: INEQUALITY OF $\dot{V}A/\dot{Q}$ RATIOS FOLLOWING SMOKE INHALATION INJURY AND THE EFFECT OF ANGIOTENSIN ANALOGUES: Time Course of $\dot{V}A/\dot{Q}$ Alterations Following Smoke Inhalation Injury in a Sheep Model

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

1 October 1985 - 30 September 1986

INVESTIGATORS

Takeshi Shimazu, MD
Avery A. Johnson, Jr., BS
Gene B. Hubbard, DVM, MS, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

PROJECT TITLE: INEQUALITY OF $\dot{V}A/\dot{Q}$ RATIOS FOLLOWING SMOKE INHALATION INJURY AND EFFECTS OF ANGIOTENSIN ANALOGUES: Time Course of $\dot{V}A/\dot{Q}$ Alterations Following Smoke Inhalation Injury in a Sheep Model

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-6200

PERIOD COVERED IN THIS REPORT: 1 Oct 85 through 30 Sep 86

INVESTIGATORS: Takeshi Shimazu, MD
Avery A. Johnson, Jr., BS
Gene B. Hubbard, DVM, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

In this report we have examined time-related alterations in $\dot{V}A/\dot{Q}$ ratio after moderate smoke inhaltion injury in a previously described sheep model. The $\dot{V}A/\dot{Q}$ change was characterized by an increase in blood flow to the low $\dot{V}A/\dot{Q}$ ($0 < \dot{V}A/\dot{Q} < 0.1$) compartment, which occurred in proportion to the severity of the inhalation injury. Increased shunt flow was not a consistent finding, although some sheep developed substantial true shunt. These changes suggest that an increase in the low VA/Q compartment is a characteristic time-related and severity-related change evoked by smoke inhalation injury.

LOW $\dot{V}A/\dot{Q}$ COMPARTMENT
SHEEP MODEL
SMOKE INHALATION INJURY
TIME-RELATED ALTERATION
$\dot{V}A/\dot{Q}$ RATIO

TIME COURSE OF $\dot{V}A/\dot{Q}$ ALTERATIONS FOLLOWING SMOKE INHALATION INJURY IN A SHEEP MODEL

INTRODUCTION

We have characterized severity-related $\dot{V}A/\dot{Q}$ alterations following smoke inhalation injury in a sheep model (1). Twenty-four hours after smoke inhalation, a significant increase of blood flow to the low $\dot{V}A/\dot{Q}$ ($0 < \dot{V}A/\dot{Q} < 0.1$) compartment in the lung was observed, while an increase of true shunt ($\dot{V}A/\dot{Q} = 0$) blood flow was not a consistant finding. In this report, we describe time-related $\dot{V}A/\dot{Q}$ alterations following smoke inhalation injury in moderately injured sheep.

MATERIALS AND METHODS

Twenty-six neutered male sheep weighing 23 to 42 (33.3 ± 4.3 = mean ± standard deviation) kilograms were used for the study. Six were studied as controls and 20 were exposed to smoke and were studied in groups of five at 6, 12, 24, and 72 hours after exposure. The smoke-exposed sheep were insufflated with an amount of smoke which, in a previous study, produced moderate inhalation injury (1-2). Cardiopulmonary indices, including $\dot{V}A/\dot{Q}$ ratios, were measured under general anesthesia, using mechanical ventilation with a fractional inspired oxygen of 0.21 as described in detail previously (1-3). Comparisons among the five groups were made by analysis of variance, using the Bonferroni correction for comparisons (4). Fractional blood flows were compared using multivariate analysis (5). Significance was assigned when $P < 0.05$.

---

[1]Shimazu T, Yukioka T, Hubbard GB, et al: Inequality of $\dot{V}A/\dot{Q}$ ratios following smoke inhalation injury and the effect of angiotensin analogues. In US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1985, pp 427-444.

[2]Shimazu T, Yukioka T, Hubbard GB, et al: A dose-responsive model of smoke inhalation injury: severity-related alteration in cardiopulmonary function. Ann Surg (in press).

[3]Shimazu T, Yukioka T, Johnson AA Jr, et al: Multiple inert gas measurement with a gas chromatography-mass spectrometer at trace level: application of a gas chromatography-mass spectrometer to the multiple inert gas elimination technique. (Submitted for publication).

[4]Dixon WJ (ed). BMDP Statistical Software. Berkeley: University of California Press, 1983.

[5]SAS® User's Guide: Statistics. Version 5. Cary, NC: SAS Institute, Inc., 1985.

RESULTS

Cardiopulmonary indices are summarized in Table 1. Significant differences between groups were found in arterial oxygen pressure, mean pulmomary arterial pressure, pulmonary resistance, and static compliance. Arterial carbon dioxide pressure, pH, mean systemic blood pressure, cardiac index, total peripheral resistance index, pulmonary vascular resistance index, and lung water, measured by a double indicator dilution method, did not reveal any significant differences.

Table 2 shows the summary of $\dot{V}A/\dot{Q}$ distribution. A significant difference was found only in the standard deviation of blood flow on a log scale (standard deviation (SD) of Q), which is an index of blood flow dispersion (6).

Blood distribution patterns are further analyzed in Table 3. Fractional blood flow to the shunt ($\dot{V}A/\dot{Q} = 0$), low $\dot{V}A/\dot{Q}$ ($0 < \dot{V}A/\dot{Q} < 0.1$), normal $\dot{V}A/\dot{Q}$ ($0.1 < \dot{V}A/\dot{Q} < 10$), and high $\dot{V}A/\dot{Q}$ ($10 < \dot{V}A/\dot{Q}$) compartments were compared using multivariate analysis. Significant difference was found in the low $\dot{V}A/\dot{Q}$ compartment and the normal $\dot{V}A/\dot{Q}$ compartment, suggesting a recruitment of blood flow from the normal $\dot{V}A/\dot{Q}$ compartment to the low $\dot{V}A/\dot{Q}$ compartment with progression of hypoxia.

DISCUSSION

The multiple inert gas elimination technique (MIGET) of measuring the matching of pulmonary blood and air flow was developed by Wagner et al in 1974 (7-8) and has been applied in both clinical and experimental settings. Half of the reported studies using MIGET have been of chronic obstructive pulmonary

---

[6]Gale GE, Torre-Bueno JR, Moon RE, et al: Ventilation-perfusion inequality in normal humans during exercise at sea level and simulated altitude. J Appl Physiol 58:978-988, 1985.

[7]Wagner PD, Saltzman HA, and West JB: Measurement of continuous distributions of ventilation-perfusion ratios: theory. J Appl Physiol 36:588-599, 1974.

[8]Wagner PD, Naumann PF, and Laravuso RB: Simultaneous measurement of eight foreign gases in blood by gas chromatography. J Appl Physiol 36:600-605, 1974.

TABLE 1

CARDIOPULMONARY INDICES (MEAN ± STANDARD DEVIATION)

	CONTROL GROUP (n = 6)	SMOKE-EXPOSED GROUP			
		6 Hours (n = 5)	12 Hours (n = 5)	24 Hours (n = 5)	72 Hours (n = 5)
Arterial Oxygen Pressure (torr)	101.0 ± 6.30	89.7 ± 9.20	78.0 ± 12.60	68.8 ± 9.20*	63.5 ± 11.60*
Arterial Carbon Dioxide Pressure (torr)	34.1 ± 4.10	32.2 ± 2.80	33.8 ± 5.10	33.8 ± 3.30	37.3 ± 9.30
Cardiac Index ($l/min/m^2$)	4.1 ± 0.72	4.8 ± 0.83	4.0 ± 0.89	4.0 ± 0.55	4.5 ± 1.40
Mean Systemic Blood Pressure (torr)	124.0 ± 8.50	123.0 ± 24.60	119.0 ± 10.70	121.0 ± 10.00	124.0 ± 8.90
Mean Pulmonary Arterial Pressure (torr)	14.1 ± 1.20	14.9 ± 4.80	18.5 ± 8.10	18.4 ± 4.70	23.4 ± 2.70**
Static Compliance (ml/cmH_2O)	180.0 ± 49.00	138.0 ± 30.00	138.0 ± 55.00	110.0 ± 27.00	75.0 ± 22.00*
Pulmonary Resistance ($cm\ H_2O\ sec/l$)	10.3 ± 2.30	12.4 ± 1.80	19.1 ± 9.80	19.4 ± 10.50	37.2 ± 14.00*
Lung Water (ml/kg)	10.2 ± 0.57	12.9 ± 2.20	15.8 ± 6.2	12.6 ± 1.10	13.4 ± 1.40

*P < 0.01 versus control group.
**P < 0.05 versus control group.

TABLE 2

VA/Q ANALYSIS (MEAN ± STANDARD DEVIATION)

	CONTROL GROUP (n = 6)	SMOKE-EXPOSED GROUP			
		6 Hours (n = 5)	12 Hours (n = 5)	24 Hours (n = 5)	72 Hours (n = 5)
Mean Q	0.903 ± 0.242	0.675 ± 0.147	0.540 ± 0.244	0.530 ± 0.255	0.483 ± 0.426
SD of Q	0.620 ± 0.055	1.021 ± 0.392	1.699 ± 0.615*	1.783 ± 0.530*	1.847 ± 0.771**
Mean $\dot{V}$	3.858 ± 1.373	4.267 ± 2.280	2.837 ± 0.785	3.556 ± 1.934	3.945 ± 1.000
$\dot{V}_D/\dot{V}_T$	0.329 ± 0.093	0.245 ± 0.081	0.315 ± 0.089	0.359 ± 0.048	0.342 ± 0.052

*P < 0.05 versus control group.
**P < 0.01 versus control group.

NOTE: These parameters are derived from the VA/Q 50-compartment lung model. Mean Q = mean blood flow (liters per minute) on log scale, SD of Q = standard deviation of blood flow on log scale (log SDq), mean V = mean ventilation (liters per minute) on log scale, and V_D/V_T = fraction of dead space ventilation. The log SDq is calculated as the square root of the second moment about the mean on a natural log scale for compartmental blood flow in the 48 VA/Q compartments other than shunt (VA/Q = 0) and dead space (VA/Q = infinity) (6). The log SDq is an index of dispersion of blood distribution pattern.

TABLE 3

FRACTIONAL BLOOD FLOW (PERCENTAGE) TO EACH COMPARTMENT (MEAN ± STANDARD DEVIATION)

	CONTROL GROUP (n = 6)	SMOKE-EXPOSED GROUP			
		6 Hours (n = 5)	12 Hours (n = 5)	24 Hours (n = 5)	72 Hours (n = 5)
$\dot{V}A/\dot{Q} = 0$ (Shunt)	0.33 ± 0.11	3.28 ± 2.18	2.18 ± 1.59	1.62 ± 1.20	3.58 ± 2.99
$0 < \dot{V}A/\dot{Q} < 0.1$ (Low)	0	3.24 ± 3.19	13.60 ± 5.70	14.90 ± 4.21	24.6 ± 9.60*
$0.1 < \dot{V}A/\dot{Q} < 10$ (Normal)	98.70 ± 0.20	92.20 ± 2.80	83.50 ± 6.80	82.50 ± 4.40	70.4 ± 7.70**
$10 < \dot{V}A/\dot{Q}$ (High)	1.06 ± 0.22	1.30 ± 0.37	0.76 ± 0.15	0.94 ± 0.45	1.38 ± 0.39

*$p < 0.05$ versus control group.
**$p < 0.01$ versus control group.

disease (9-13); others have dealt with acute respiratory failure, including smoke inhalation injury (14), oleic acid injury (15-17), hemorrhage (18), gas emboli (19), lung edema (17), adult respiratory distress syndrome (20), anesthesia

---

[9]Wagner PD, Dantzker DR, Dueck R, et al: Ventilation-perfusion inequality in chronic obstructive pulmonary disease. J Clin Invest 59:203-216, 1977.

[10]Wagner PD, Dantzker DR, Iacovoni VE, et al: Ventilation-perfusion inequality in asymptomatic asthma. Am Rev Res Dis 118:511-524, 1978.

[11]Rubinfeld AR, Wagner PD, and West JB: Gas exchange during acute experimental canine asthma. Am Rev Respir Dis 118:525-536, 1978.

[12]Rodriguez-Roisin R, Bencowitz HZ, Ziegler MG, et al: Gas exchange responses to bronchodilators following methacholine challenge in dogs. Am Rev Respir Dis 130:617-626, 1984.

[13]Corte P and Young IH: Ventilation-perfusion relationships in symptomatic asthma. Response to oxygen and clemastine. Chest 88:167-175, 1985.

[14]Robinson NB, Hudson LD, Robertson HT, et al: Ventilation and perfusion alterations after smoke inhalation injury. Surgery 90:352-363, 1981.

[15]Shoene RB, Robertson HT, Thorning DR, et al: Pathophysiological patterns of resolution from acute oleic acid lung injury in the dog. J Appl Physiol 56:472-481, 1984.

[16]Wagner PD, Laravuso RB, Goldzimmer E, et al: Distributions of ventilation-perfusion ratios in dogs with normal and abnormal lungs. J Appl Physiol 38:1099-1109, 1975.

[17]Derks CM, D'Hollander AA, and Jacobovitz-Derks D: Gas exchange and respiratory mechanics in moderate and severe pulmonary oedema in dogs. Bull Europ Physiopathol Respir 17:163-177, 1981.

[18]Robinson NB, Chi EY, and Robertson HT: Ventilation-perfusion relationships after hemorrhage and resuscitation: an inert gas analysis. J Appl Physiol 54:1131-1140, 1983.

[19]Hlastala MP, Robertson HT, and Ross BK: Gas exchange abnormalities produced by venous gas emboli. Res Physiol 36:1-17, 1979.

[20]Dantzker DR, Brook CJ, Dehart P, et al: Ventilation-perfusion distributions in the adult respiratory distress syndrome. Am Rev Respir Dis 120:1039-1052, 1979.

(21-22), carbon monoxide poisoning (23), meconium aspiration (24), and pulmonary embolism and pneumonia (16). One of the major advantages of MIGET is its ability to differentiate quantitatively between true shunt ($\dot{V}A/\dot{Q} = 0$) and low $\dot{V}A/\dot{Q}$ ($0 < \dot{V}A/\dot{Q} < 0.1$) compartments, which are indistinguishable by conventional venous admixture measurements using the Berggren equation. In the reports of acute respiratory insufficiency, true shunt and low $\dot{V}A/\dot{Q}$ compartments make variable degrees of contribution to the resultant hypoxia, reflecting the specific pathophysiology of the disease. Increased true shunt was observed in oleic acid-induced lung edema with minimal or no increase in low $\dot{V}A/\dot{Q}$ areas (15-17). Shoene et al reported that oleic acid-injured dogs developed significant shunt at day 1 with resolution of the shunt by day 7, while animals that developed suppurative bronchopneumonia induced by repeated bronchial lavage showed low $\dot{V}A/\dot{Q}$ peaks (15). Gas emboli produced hypoxia mainly attributable to low $\dot{V}A/\dot{Q}$ areas (19). Other acute injuries showed combinations of shunt and low $\dot{V}A/\dot{Q}$ areas. Robinson et al have reported an early (within 24 hours of injury) increase in high $\dot{V}A/\dot{Q}$ and dead space compartments and a late (after 48 hours) increase in low $\dot{V}A/\dot{Q}$ compartments in burn patients with smoke inhalation (14).

In this report, we have demonstrated that time-related $\dot{V}A/\dot{Q}$ change following smoke inhalation injury was characterized by development of a low $\dot{V}A/\dot{Q}$ compartment, accounting for the progressive hypoxia after injury. Increased blood flow to a true shunt was not a consistant finding. These time-related $\dot{V}A/\dot{Q}$ alterations are identical to those severity-related $\dot{V}A/\dot{Q}$ alterations that we have reported previously (1). Robinson et al reported that in a rabbit model of smoke inhalation injury there was no increase in the low $\dot{V}A/\dot{Q}$ compartment, but there was broadening of the $\dot{V}A/\dot{Q}$ peak centered at $\dot{V}A/\dot{Q} = 1$ at six hours postinjury when arterial oxygen pressure was still maintained (14). We observed the same change as reported in a previous study in sheep exposed to less severe smoke injury than in this study (1). Since we did not study the time course

---

[21]Dueck R, Rathbun M, and Wagner PD: Chromatographic analysis of multiple tracer inert gases in the presence of anesthetic gases. Anesthesiology 49:31-36, 1978.

[22]Dueck R, Young I, Clausen J, et al: Altered distribution of pulmonary ventilation and blood flow following induction of inhalation anesthesia. Anesthesiology 52:113-125, 1980.

[23]Robinson NB, Barie PS, Halebian PH, et al: Distribution of ventilation and perfusion following acute carbon monoxide poisoning. Surg Forum 36:115-118, 1985.

[24]Truog WE, Lyrene RK, Standaert TA, et al: Effects of PEEP and tolazoline infusion on respiratory and inert gas exchange in experimental meconium aspiration. J Pediatr 100:284-290, 1982.

of those with milder smoke inhalation injury, we do not know the natural history of that broadened $\dot{V}A/\dot{Q}$ peak. We have now demonstrated transition of the broadened peaks (unimodal distribution) to a low $\dot{V}A/\dot{Q}$ compartment (bimodal distribution).

Although MIGET is a very useful method for the study of the pathophysiology of respiratory insufficiency, there is no standard mathematical method of analyzing and comparing the observed $\dot{V}A/\dot{Q}$ distributions. This results from the nature of MIGET, i.e., MIGET is not a unique solution or eigenvalue, but one of many solutions that satisfies inert gas elimination data in a parallel 50-compartment lung model (7-8,25). As Hlastala et al (25) has pointed out, we can not treat the recovered $\dot{V}A/\dot{Q}$ distribution curve as an absolute entity.

There are two kinds of comparisons commonly used to analyze physiologic determinations, first, comparison between various groups, and second, comparison between repeated measurements in the same subject. Kapitan et al (26) have recently developed a linear programming method for comparisons of the latter type. For the former type of comparison, it is fairly common to divide $\dot{V}A/\dot{Q}$ compartments into five major parts, the shunt ($\dot{V}A/\dot{Q} = 0$), low $\dot{V}A/\dot{Q}$ ($0 < \dot{V}A/\dot{Q} < 0.1$), normal $\dot{V}A/\dot{Q}$ ($0.1 < \dot{V}A/\dot{Q} < 10$), high $\dot{V}A/\dot{Q}$ ($10 < \dot{V}A/\dot{Q}$), and dead space fractions as are shown in Table 3 (9,13-15,24). Another common way is to compare values of shunt flow, mean blood flow on log scale (mean Q, Table 2), mean log SD of blood flow (SD of Q, Table 2), and mean ventilation and log SD of ventilation (13,25).

Whichever comparison is used, it is questionable to use the means and standard deviation (SD) of the indices in order to obtain representative values of a group and make comparisons between groups. We cannot use a separate univariate analysis of each dependent (index) in this situation because each of those indices, i.e., blood flows to the shunt, low $\dot{V}A/\dot{Q}$, normal $\dot{V}A/\dot{Q}$, and high $\dot{V}A/\dot{Q}$ fractions or percent flow to shunt, mean $\dot{Q}$ and log SD of $\dot{Q}$, are not independent of one another. For example, a decrease in one fraction will result in an increase in other fraction(s) if the cardiac output does not change significantly. Similarly, a decrease in shunt flow will result in lower mean $\dot{V}A/\dot{Q}$ and/or larger log SD of $\dot{Q}$ if arterial oxygen pressure did not change significantly. Univariate analysis in that situation will cause the probability of a Type I error to be higher for each analysis than the level of significance that is used and the probability of finding a significant

---

[25]Hlastala MP: Multiple inert gas elimination technique. J Appl Physiol 56:1-7, 1984.

[26]Kapitan KS and Wagner PD: Linear programming analysis of VA/Q distributions: limits on central moments. J Appl Physiol 60:1772-1781, 1986.

difference by chance alone increases as the number of dependent variables (indices) increases (27). For this reason, it would be better to use a multivariate analysis for comparison between groups. However, there might still be a problem related to the control values (normality). Normal subjects do not have shunt or low $\dot{V}A/\dot{Q}$ fractions and the values for these are all 0, i.e., the normal animals do not have a normal distribution in blood flow to the low $\dot{V}A/\dot{Q}$ compartment.

In conclusion, time-related $\dot{V}A/\dot{Q}$ alteration was characterized by increased low $\dot{V}A/\dot{Q}$ compartment but an increase in true shunt was not a consistent finding. These changes are identical to those observed as injury/severity-related changes in previous studies.

PRESENTATIONS/PUBLICATIONS

None.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Dr. Peter Westfall, PhD, of the University of Texas Health Science Center at Lubbock, Texas, for his helpful suggestions in the statistical treatment of $\dot{V}A/\dot{Q}$ data.

[27]Huck SW, Cormier WH, and Bounds WG: Reading Statistics and Research. New York: Harper & Row Publishers, 1974.

PRESENTATIONS

Pruitt BA Jr: Diagnosis and treatment of infection in severely injured patients. Presented at the Physicians Forum, IGIV Conference, Houston, Texas, 1 October 1985.

Latona PS: An overview of the Nursing Service Branch at the US Army Institute of Surgical Research. Presented to the Practical Nurse Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 4 October 1985.

Vaughan GM: Nyctohemeral rhythm and light sensitivity of a Syrian hamster pineal melatonin response to norepinephrine. Presented at the Second Annual Army-ACP Regional Meeting, San Francisco, California, 6-11 October 1985.

Roberts LW: Burns. Presented to the Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 16 October 1985.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in injured man. Presented to the Department of Surgery, University of Vermont, Burlington, Vermont, 24 October 1985.

Pruitt BA Jr: Future concepts of the care of military burn patients. Presented at the Armed Forces Radiobiology Research Institute, Bethesda, Maryland, 25 October 1985.

Cozean RJ: Psychosocial aspects of care and management of the burn patient. Presented at the Third Annual Military Health Professionals Symposium, 2290th US Army Hospital, Rockville, Maryland, 26 October 1985.

Kyzar DW: Comprehensive nursing care for the burn-injured patient. Presented at the Third Annual Military Health Professionals Symposium, 2290th US Army Hospital, Rockville, Maryland, 26 October 1985.

McCoy KF: Role of physical therapy and occupational therapy in burn rehabilitation. Presented at the Third Annual Military Health Professionals Symposium, 2290th US Army Hospital, Rockville, Maryland, 26 October 1985.

McManus WF: Resuscitation and early care of the burn patient. Presented at the Third Annual Military Health

Professionals Symposium, 2290th US Army Hospital, Rockville, Maryland, 26 October 1985.

Pruitt BA Jr: Care and closure of the burn wound. Presented at the Third Annual Military Health Professionals Symposium, 2290th US Army Hospital, Rockville, Maryland, 26 October 1985.

Pruitt BA Jr: Epidemiology, first aid triage, and aeromedical transfer of the burn casualty. Presented at the Third Annual Military Health Professionals Symposium, 2290th US Army Hospital, Rockville, Maryland, 26 October 1985.

Gutierrez RT: Care of the thermally injured patient. Presented to the Physical Therapy Specialist Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 30 October 1985.

Zellers LA: Transport of the burn victim. Presented to the Aviator Course (2CF7), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 30 October 1985.

Gutierrez RT: Physical therapy in burns. Presented to the Physical Therapy School students from Southwest Texas State University, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 4 November 1985.

McCoy KF: Physical therapy in burns. Presented to the Physical Therapy School students from Southwest Texas State University, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 4 November 1985.

Pruitt BA Jr: Current treatment of the burn wound. Presented at the Waterbury Hospital, Waterbury, Connecticut, 7 November 1985.

Vaughan GM: Human melatonin in physiologic and diseased states: neural control of the rhythm. Presented at the Symposium on Melatonin in Humans, Vienna, Austria, 7-9 November 1985.

Latona PS: Initial management of the burn victim. Presented to the Reserve Nurses, 94th General Army Reserve Unit, Fort Hood, Texas, 9 November 1985.

Jordan BS: Wound management of the burn victim. Presented to the Reserve Nurses, 94th General Army Reserve Unit, Fort Hood, Texas, 9 November 1985.

Pruitt BA Jr: Planning and design of burn centers. Presented at the Third Annual Harvey A. Beffa Shriners Burn Institute Conference, Galveston, Texas, 10-12 November 1985.

Kyzar DW: An overview of the Nursing Service Branch at the US Army Institute of Surgical Research. Presented to the Nurse Educator Tour, US Army Recruiting Command, Fort Sam Houston, San Antonio, Texas, 13 November 1985.

Luster SH: Rehabilitation of the burn-injured veteran. Presented at the Annual Meeting of the Association of Military Surgeons of the United States, Anaheim, California, 13 November 1985.

Wilson SW: Burn information system - nutrition module. Presented to the Symposium on Computer Applications in Medical Care, Baltimore, Maryland, 13 November 1985.

McManus WF: Electrical burns. Presented at the Partnerships Beyond Survival Seminar, Baptist Medical Center, Oklahoma Medical Center, Oklahoma City, Oklahoma, 14-15 November 1985.

McManus WF: Emergency care, triage, evacuation, and fluid resuscitation. Presented at the Partnerships Beyond Survival Seminar, Baptist Medical Center, Oklahoma Medical Center, Oklahoma City, Oklahoma, 14-15 November 1985.

McManus WF: Infection control in the burn center. Presented at the Partnerships Beyond Survival Seminar, Baptist Medical Center, Oklahoma Medical Center, Oklahoma City, Oklahoma, 14-15 November 1985.

Pruitt BA Jr: Care and coverage of the burn wound. Presented at the Burn Center Symposium, Baptist Medical Center, Oklahoma City, Oklahoma, 14-15 November 1985.

Pruitt BA Jr: Inhalation injury and pulmonary complications. Presented at the Burn Center Symposium, Baptist Medical Center, Oklahoma City, Oklahoma, 14-15 November 1985.

Cozean RJ: Psychosocial aspects of the burn victim. Presented to the Alabama State Nurses Association, Southeast Recruiting Command, 20 November 1985.

Jordan BS: Wound management of the burn victim. Presented to the Alabama State Nurses Association, Southeast Recruiting Command, 20 November 1985.

Latona PS: Initial management of the burn victim. Presented to the Alabama State Nurses Association, Southeast Recruiting Command, 20 November 1985.

Culbertson GR: Burns. Presented to the Special Operations Medical Sergeants Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 2 December 1985.

Latona PS: Initial management of the burn victim. Presented to the Luling Texas Paramedic Association, Luling, Texas, 5 December 1985.

Zellers LA: Initial management of the burn victim. Presented to the Luling Texas Paramedic Association, Luling, Texas, 5 December 1985.

Pruitt BA Jr: Immunologic responses to burn injury. Presented at the Fifth Annual Comprehensive Care of the Burn Patient Conference, Kansas City, Kansas, 6-7 December 1985.

Pruitt BA Jr: Pathophysiology of inhalation injury. Presented at the Fifth Annual Comprehensive Care of the Burn Patient Conference, Kansas City, Kansas, 6-7 December 1985.

Wilson SW: Nutritional management of the burn patient. Presented to Dietetic Students, Incarnate Word College, San Antonio, Texas, 10 December 1985.

Kyzar DW: An overview of the Nursing Service Branch at the US Army Institute of Surgical Research. Presented to the Army Medical Department Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 December 1985.

Latona PS: Burn patient management. Presented to the Aviator Course (2CF7), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 December 1985.

Latona PS: Critical care. Presented to the Short Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 13 December 1985.

Luster SH: Occupational therapy in burn rehabilitation. Presented to the Occupational Therapy Assistant Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 7 January 1986.

Roberts LW: Burns. Presented to the Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 14 January 1986.

Pruitt BA Jr: The hemodynamic response to burn injury and resuscitation. Presented at the Carnegie-Mellon University Research Seminar, Pittsburgh, Pennsylvania, 16 January 1986.

Pruitt BA Jr: Epidemiology and pathophysiology of burn injury. Presented at the US Army Institute of Surgical Research OT/PT Short Course, Fort Sam Houston, San Antonio, Texas, 21 January 1986.

Gutierrez RT: Physical therapy in burn care. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Fort Sam Houston, San Antonio, Texas, 21-31 January 1986.

McCoy KF: Biomechanical complications of thermal injury. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Fort Sam Houston, San Antonio, Texas, 21-31 January 1986.

McManus WF: Management of burns in the theater of operations. Presented at the OT/PT Conference on Management of Burns in the Theater of Operations, Fort Sam Houston, San Antonio, Texas, 21-31 January 1986.

Wilson SW: Nutritional management of the burn patient. Presented to the Dietetic Interns, Baptist Memorial Hospital, San Antonio, Texas, 28 January 1986.

Culbertson GR: Burns. Presented to the Special Operations Medical Sergeants Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 30 January 1986.

Pruitt BA Jr: Metabolic management of burn patients. Presented as Visiting Professor at the Albany Medical College, Albany, New York, 5-7 February 1986.

McManus WF: Management of burns. Presented to the Flight Surgeons, Randolph Air Force Base, Texas, 10 March 1986.

Pruitt BA Jr: Opportunistic infections in injured patients. Presented as Visiting Professor to the Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, California, 12 March 1986.

Gutierrez RT: Physical therapy and occupational therapy of burns. Presented to the Advanced Physical Therapy Course from Wilford Hall Medical Center, Fort Sam Houston, San Antonio, Texas, 18 March 1986.

McManus WF: Pathophysiology and anatomy of the burn wound. Presented to the USAF Advanced Physical Therapy Course, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 18 March 1986.

Pruitt BA Jr: Fluid resuscitation of the burn patient. Presented as Visiting Professor at the University of Illinois College of Medicine, Champaign, Illinois, 20-21 March 1986.

Pruitt BA Jr: Infection and burn problems. Presented to the Physician's Assistant Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 March 1986.

McManus WF: Assessment and management of the burn patient. Presented at the Second Annual Tri-State Trauma Symposium, Lubbock, Texas, 28 March 1986.

Pruitt BA Jr: The burn patient as the universal trauma model. Presented as Visiting Professor at the Albany Medical College, Albany, New York, 1-2 April 1986.

Pruitt BA Jr: Infection/drugs/topical therapy. Presented at the Eighteenth Annual Meeting of the American Burn Association, Chicago, Illinois, 11 April 1986.

Shirani KZ: The influence of inhalation injury and pneumonia on burn mortality. Presented at the Eighteenth Annual Meeting of the American Burn Association, Chicago, Illinois, 11 April 1986.

Pruitt BA Jr: Infection and burn problems. Presented to the Physician's Assistant Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 16 April 1986.

Jordan BS: Team effort with burn care management. Presented at the INTRACORP Seminar, Dallas, Texas, 1-2 May 1986.

McCoy KF: Costs of physical therapy/occupational therapy in burn care. Presented at the INTRACORP Seminar, Dallas, Texas, 1-2 May 1986.

McCoy KF: Team effort with burn care management. Presented at the INTRACORP Seminar, Dallas, Texas, 1-2 May 1986.

Missavage AE: Team effort with burn care management. Presented at the INTRACORP Seminar, Dallas, Texas, 1-2 May 1986.

Pruitt BA Jr: Current management of the burn patient. Presented as Visiting Lecturer to the North Dakota Chapter of the American College of Surgeons and the North Dakota Medical Society, Grand Forks, North Dakota, 1-2 May 1986.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in injured man. Presented as Visiting Lecturer to the North Dakota Chapter of the American College of Surgeons and the North Dakota Medical Society, Grand Forks, North Dakota, 1-2 May 1986.

McManus WF: Resuscitation of thermal, chemical, and electric-injured patients. Presented to the 1986 Military Medical/Surgical Congress, Garmisch, West Germany, 6-9 May 1986.

Cozean KL: Occupational therapy in burn rehabilitation. Presented to the Occupational Therapy Assistant Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 9 May 1986.

Pruitt BA Jr: Infection and burn problems. Presented to the Physician's Assistant Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 21 May 1986.

Roberts LW: Burns. Presented to the Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 6 June 1986.

Roberts LW: Burns. Presented to the Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 20 June 1986.

Pruitt BA Jr: Individualized fluid resuscitation of the burn patient. Presented as Invited Speaker to the 50th Annual Anniversity Course, Trauma and Critical Care Surgery, University of Minnesota, Minneapolis, Minnesota, 20-21 June 1986.

Pruitt BA Jr: Burns during pregnancy. Presented as Invited Speaker to the Burns Management Course, New Delhi, India, 23 June through 4 July 1986.

Pruitt BA Jr: Chemical burns. Presented as Invited Speaker to the Burns Management Course, New Delhi, India, 23 June through 4 July 1986.

Pruitt BA Jr: Local burn wound infection. Presented as Invited Speaker to the Burns Management Course, New Delhi, India, 23 June through 4 July 1986.

Pruitt BA Jr: Inhalation burns. Presented as Invited Speaker to the Burns Management Course, New Delhi, India, 23 June through 4 July 1986.

Pruitt BA Jr: The Systemic Response to burn injury - pathophysiology. Presented as Invited Speaker to the Burns Management Course, New Delhi, India, 23 June through 4 July 1986.

McCoy KF: Physical therapy in burn care. Presented to the US Army-Baylor Physical Therapy Program, Fort Sam Houston San Antonio, Texas, 24 June 1986.

Roberts LW: Burns. Presented to the Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 24 June 1986.

McCoy KF: Care of the thermally injured patient. Presented to the Physical Therapy Specialist Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 25 June 1986.

Roberts LW: Burns. Presented to the Officers' Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 15 July 1986.

Pruitt BA Jr: Infection: cause or effect of pathophysiologic change in burn and trauma patients. Presented as participant in the NATO-Sponsored Seminar on Lipid Mediators and Immunology in Trauma, Helsingor, Denmark, 20-25 July 1986.

Pruitt BA Jr: Treatment of thermal injuries. Presented as Visiting Professor to the Department of Surgery, University of South Alabama College of Medicine, Mobile, Alabama, 8 August 1986.

Cozean KL: Elastomier pressure devices in control of scar tissue. Presented to the Orthopedic Hand Service, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 14 August 1986.

Pruitt BA Jr: The history of animal researchd. Presented at the Harvey Beffa Conference, Shriners Hospital for Crippled Children, Cincinnati, Ohio, 7-9 September 1986.

Luster SH: Occupational therapy in burn rehabilitation. Presented to the Occupational Therapy Assistant Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 9 September 1986.

Pruitt BA Jr: The burn patient as a trauma model. Presented as the Curtis Artz Lecturer to the South Carolina Chapter of the American College of Surgeons, Greenville, South Carolina, 12-13 September 1986.

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

PUBLICATIONS

Shirani KZ, Vaughan GM, Pruitt BA Jr, and Mason AD Jr: Reduced serum T_4 and T_3 and their altered serum binding after burn injury in rats. J Trauma 25(10):953-958, October 1985.

Kim SH, Hubbard GB, McManus WF, Mason AD Jr, and Pruitt BA Jr: Frozen section technique to evaluate early burn wound biopsy: a comparison with the rapid section technique. J Trauma 25(12):1134-1137, December 1985.

Pruitt BA Jr: The diagnosis and treatment of infection in the burn patient. Burns Incl Therm Inj 11(2):79-91, December 1985.

Scott DE, Vaughan GM, and Pruitt BA Jr: Hypothalamic neuroendocrine correlates of cutaneous burn injury in the rat: I. Scanning electron microscopy. Brain Res Bull 17(3):367-378, September 1986.

Burleson DG: Clinical applications of flow cytometry (abstract). Proc Soc Armed Forces Mil Lab Sci 14:17, 1985.

Burleson DG, Vaughan GK, and Mason AD Jr: Differences in the measurement of human neutrophil respiratory burst oxidative activity using molecular probes. Fed Proc (Abstract 989) 44:580, 1985.

Pruitt BA Jr, Goodwin CW, Vaughan GM, Aulick LH, Newman JJ, Strome DR, and Mason AD Jr: The metabolic problems of the burn patient. Acta Chir Scad (Suppl) 522:119-139, 1986.

Pruitt BA Jr, Mason AD Jr, McManus WF, Bush KW, and Hoffman BE: Burn injury: panel presentation. Trans Assoc Life Insur Med Dir Am 67:158-186, 1985.

Vaughan GM, Lasko J, Coggins SH, Pruitt BA Jr, and Mason AD Jr: Rhythmic melatonin response of the Syrian hamster pineal gland to norepinephrine *in vitro* and *in vivo*. J Pineal Res 3(3):235-249, 1986.

Vaughan GM, Mason AD Jr, and Reiter RJ: Serum melatonin after a single aqueous subcutaneous injection in Syrian hamsters. Neuroendocrinol 42:124-127, 1985.

Vaughan GM and Pruitt BA Jr: Pineal-induced depression of free thyroxine in Syrian hamsters. J Pineal Res 2(4):325-330, 1985.

Vaughan GM, Pruitt BA Jr, Shirani KZ, Vaughan MK, and Mason AD Jr: The thyroid axis and brain 5'-monodeiodination of thyroxine in the burned rat model of nonthyroidal illness. Neuroendocrinol Lett 8(5):221-226, 1986.

Vaughan GM, Shirani KZ, Ziegler MG, Pruitt BA Jr, and Mason AD Jr: The burned hamster as a model of nonthryoidal illness. Proceedings of the 67th Annual Meeting of the Endocrine Society (Abstract 1281):321, 1985.

Vaughan GM, Taylor TJ, Lasko J, Bullard JJD, Coggins SH, Brown AC, and Mason AD Jr: The human melatonin rhythm and lack of influence by sympathetic activation. Adv Biosci 53:37-40, 1985.

Vaughan GM, Taylor TJ, Pruitt BA Jr, and Mason AD Jr: Pineal function in burns: melatonin is not a marker for general sympathetic activity. J Pineal Res 2(1):1-12, 1985.

Vaughan GM, Vaughan MK, and Mason AD Jr: Correlation of thyroid hormones and testes weight across pineal-related perturbations. Adv Biosci 53:257-264, 1985.

Shirani KZ, McManus AT, Vaughan GM, McManus WF, Pruitt BA Jr, and Mason AD Jr: Effects of environment on infection in burn patients. Arch Surg 121(1):31-36, January 1986.

Pruitt BA Jr: Host-opportunistic interactions in surgical infection. Arch Surg 121(1):13-22, January 1986.

Yukioka T, Yukioka N, Aulick LH, Goodwin CW, Mason AD Jr, Sugimoto T, and Pruitt BA Jr: Evaluation of (1-sarcosine, 8-isoleucine) angiotensin II as a therapeutic agent for oleic acid-induced pulmonary edema. Surgery 99(2):235-244, February 1986.

Kingsley CP, Schmidt SI, and Reynolds WJ: A method for securing "piggyback" infusions (letter). Anesth Analg 65(2):209-210, 1986.

Missavage AE, McManus WF, and Pruitt BA Jr: Suppurative thrombophlebitis. In Current Therapy in Vascular Surgery. Ernest CB and Stanley JS (eds). Burlington: BD Decker, Inc., 1986.

Vaughan GM and Reiter RJ: Pineal dependence of the Syrian hamster's nocturnal serum melatonin surge. J Pineal Res 3(1):9-14, 1986.

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-6200

MOTION PICTURES

Pruitt BA Jr and McManus WF: Critical Decisions in Surgery #2: Management of Severe Burns. New York: Park Row Publishers, 1986.

DISTRIBUTION LIST

Number of Copies	
5	Commander US Army Medical Research and Development Command ATTN: SGRD-RMI-S Fort Detrick Frederick, Maryland 21701-5012
1	Commander Letterman Army Institute of Research Building 1110 Presidio of San Francisco, California 94129-6800
1	Director Walter Reed Army Institute of Research Building 40 Washington, DC 20307-5100
1	Commander US Army Aeromedical Research Laboratory Building 8708 Fort Rucker, Alabama 36362-5292
1	Commander US Army Institute of Dental Research Building 40 Washington, DC 20307-5300
1	Commander US Army Medical Biomedical Research and Development Laboratory Building 568 Fort Detrick Frederick, Maryland 21701-5010
1	Commander US Army Medical Research Institute of Chemical Defense Building E3100 Edgewood Avenue Aberdeen Proving Ground, Maryland 21010-5425
1	Commander US Army Medical Research Institute of Infectious Diseases Building 1425 Fort Detrick Frederick, Maryland 21701-5011

Number of Copies	
1	Commander US Army Research Institute of Environmental Medicine Building 42 Natick, Massachusetts 01760-5007
1	Defense Technical Information Center ATTN: DTIC-DDA Alexandria, Virginia 22304-9990
1	Commandant Academy of Health Sciences ATTN: AHS-CDM Fort Sam Houston, Texas 78234
1	Director Biological and Medical Sciences Division Office of Naval Research 800 North Quincy Street Arlington, Virginia 22217
1	Commanding Officer Naval Medical Research and Development Command National Naval Medical Center Bethesda, Maryland 20014
1	Headquarters United States Air Force Medical Service Center ATTN: Chief, Aerospace Medical Consultant Division Building 150 (SGPA) Brooks Air Force Base, Texas 78255
1	Director of Defense Research and Engineering ATTN: Assistant Director Environmental and Life Sciences Washington, DC 20301

* U.S. GOVERNMENT PRINTING OFFICE 1988 571-501 85011